THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME I MARCH, 1925 NUMBER 2

EVIDENCE OF REGENERATION OF PANCREAS IN AN INSULIN TREATED CASE OF DIABETES *

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The peculiar pathology of the pancreas renders a knowledge of its histology essential to the understanding of any disease process involving it. The absence of any gross changes in the pancreas in many cases of diabetes has led to much confusion respecting the pathology of this disease and to some doubt of its being of pancreatic origin. Current literature tends to the view that changes are present when a close study of the histopathology of the organ is made.

Early in embryonic life three buds from the primitive intestinal tract develop, two ventral (Santorini) and a dorsal one (Wirsung). By the end of the second month of fetal life these anlagen fuse and give rise to all the structures of the adult pancreas. Pearce [1] and Küster [2] note separation and vascularity of cell groups in the third month. Such groups consist of four more or less distinct types of tissue — the ducts, centro-acinar cells, islet cells, and acinar cells. These structures are arranged in lobes separated by small amounts of connective tissue and again subdivided into lobules by the ramifications of the ducts. A typical lobule consists of a semi-spherical mass of cells into the centre of which a duct enters. In close proximity to the duct and possibly arising from it are numbers of flattened clear cells, the centro-acinar cells. The remainder of the lobule consists of pyramid-shaped acinar cells whose apices are

* Read at the Second Annual Meeting of the Canadian Society for the Study of Diseases of Children, at Toronto, June 14, 1924. Received for publication October 17, 1924.

filled with zymogenous granules, and the so-called islands of Langerhans. These were first described by Langerhans[3] in 1869 as small groups of cells studding the pancreas and differentiated from the acinar cells by their clarity and lack of zymogen granules. As early as 1886, Lewaschew[4] observed the presence of two types of cells in these islets differentiated by their shape and mode of delimitation. Ten years later Laguesse[5] described the presence of safranophile granules resembling zymogen granules but finer and more resistant to acetic acid. From this period until 1907 when Lane's work appeared, little progress was made in the study of the histopathology of the pancreas, the literature during this period being chiefly concerned with the origin of islets and a discussion as to their being a histological entity at all. The work of Lane[6] and Bensley,[7] through elaboration of technique and stains, has made possible a much clearer understanding of the composite cells of the islets and has shown that the early workers, because of their inability to differentiate clearly the acinar and islet cells, had given a wrong interpretation of their nature. These workers showed the presence of two, and possibly three, types of cells in an islet, the so-called A and B cells, and a third less differentiated Gamma cell. The cells of the islet are typically arranged in cords, but in many instances solitary islet cells are noted among a group of acinar cells. Some of these it is claimed are the result of artifacts. Islets are frequently found in close association with ducts but never actually communicate with them. This fact and their great vascularity led to the belief that these cells produced an internal secretion.

The frequent lack of gross pathological change in the pancreas has been a stumbling block to the acceptance of the theory of the pancreatic origin of the disease. The so-called simple atrophy of the pancreas with diminution in the number of islets alone, and accompanied in older patients by interacinar fibrosis, has been reported often. Pleasants[8] in 1900 described six cases of this type in children, considering it an evidence of congenital lack of development of these structures. A little later Ssobolew[9] reported fifteen such cases, in four of which no islets at all were observed. Failure of these observers to make serial sections and the lack of suitable staining technique may explain many of these negative findings. Hydropic degeneration of the islet cells, now regarded as pathognomonic of diabetes, was first described by Weichselbaum and

Stangl [10] in 1911, and shortly after, Homans [11] showed that these changes were confined to the B cells. Allen [12] has recently made elaborate studies of this type of degeneration and the factors influencing its development. He found definite vacuolation of the cells within five days of partial pancreatectomy. The remaining islet cells rapidly degnerate after this operation and in six weeks to two months the B cells are completely destroyed and replaced by acinar tissue with small groups of A cells interspersed. While under these conditions, the majority of vacuolated cells die; this may be prevented by the avoidance of over-function by means of diet and may allow recovery to occur. Such islands always remain small and may readily be overlooked without the use of suitable stains, giving the appearance of simple atrophy with decrease in the number of islets. Allen has also shown that active diabetes is a prerequisite to the development of these changes.

The following case was found suitable for reporting because of the clinical response to insulin associated with anatomical changes strongly suggestive of regeneration in the pancreas.

Clinical History: B. N. white male — age 9.

Family History: Father and one maternal uncle both have diabetes.

Diabetes was diagnosed in this child when he was two years old. He was placed on a suitable Allen diet, which was strictly adhered to, and for a time did well except for recurrent attacks of dysentery, which lowered his tolerance. Failure to gain in stature or weight in any way commensurable with his age was noted and the general conditon became worse each year until he was more or less a chronic invalid with increasingly frequent attacks of acidosis during the last year before starting insulin.

He was admitted to the Hospital for Sick Children, Toronto, the end of December 1922. At this time he was an emaciated dwarf, more or less drowsy and unhappy. His weight was 30 pounds and his height 39 inches. His tolerance to carbohydrate had decreased until he was now unable to utilize 15 grams of such food. Insulin treatment was started at once and his diet increased to a diet suitable for a boy of his age, sufficient insulin being given to keep him sugar free and his blood sugar normal. He was discharged on an adequate diet plus insulin. Progress both in general condition and in improvement of pancreatic function was steady.

The accompanying chart shows his decreasing need of insulin with his gain in weight. His tolerance to carbohydrate trebled in the year as shown either by the fact that 30 units of insulin controlled the disease as adequately as 90 a year before, or, stated in another way, without insulin he could now handle 45 grams of carbohydrate in place of 15.

The photographs taken when insulin was started and six months later indicated in some measure the improvement in general condition. From a chronic invalid in 1922 he became the leader of the "gang" in 1923. He was killed by

fracturing his skull when sleigh-riding. He lived only about three hours after receipt of the injury and an immediate post mortem examination was made.

Autopsy Findings: The pancreas, which was removed within thirty minutes of death, was immediately cut into small blocks, labeled and portions from the head, body, and tail placed in a 10-per-cent solution of neutral formalin and also in Zenker's fluid with acetic acid. The blocks were embedded in paraffin and sections from 2–6 microns were cut.

The formalin-fixed sections were stained in Ehrlich's hematoxylin and eosin. Some formalin-fixed material was further fixed in Bensley's acetic-osmic-bichromate mixture as for fresh tissue and later stained with Altmann's anilin acid fuchsin and methyl green. Cresyl violet was also used with some of this latter material to bring out nuclear contents more clearly.

The Zenker-fixed preparations were stained with a variety of special stains to show up their specific granules. The stains used were Bensley's neutral gentian violet, Martin's azo-fuchsin and basic ethyl violet, Bensley's safranin-acid violet, Altmann's acid fuchsin and methyl green, Mallory's phosphotungstic acid hematoxylin, and finally a stain by Bowie, to whom we are indebted for the privilege of using the method before publication, namely Biebrich scarlet and basic ethyl violet. This latter stain we found very satisfactory as a differential stain showing up as it does, with the acid Zenker's fixative, the granules of the A cells blue and those of the B cells red.

The pancreas in the gross, as in so many cases of diabetes, presented nothing abnormal. It weighed twenty-nine grams, which, considering the age of the patient and his condition, was good weight. Its color, shape, lobulation, and consistency appeared normal. On gross section nothing indicating a fibrosis, adipose replacement, fat necrosis, or hemorrhagic necrosis could be detected.

On histological examination of sections from different areas we were unable to find any distinctive lesions such as hydropic degeneration of islet cells, fibrosis or atrophy of the islets as are commonly described associated with diabetes. Neither could we detect associated lesions such as inter- and intra-lobular fibrosis, chronic pancreatitis, simple atrophy, or adipose replacement. The number and size of the islets gave no indication of the existence of a possible diabetes. The only finding was the presence of occasional pyknotic

nuclei in some of the islet cells. The negative findings just mentioned, however, are not inconsistent with diabetes. This has been pointed out by many observers and serves to prove that functional capacity of the islet cells, as determined by clinical laboratory tests, is after all the final criterion for determining the ability of the individual to utilize carbohydrates. With our present facilities we are not able histologically to estimate function and we must therefore rely upon clinical tests for this determination. While clinically, in this case, there was a marked rise in sugar tolerance, it never returned to normal and the case therefore remained one of diabetes. The rise in sugar tolerance would indicate either that the islets were not irreparably injured, or if so, that regeneration had taken place in others to compensate for the loss.

While nothing in the nature of a retrograde process was found in this pancreas beyond the presence of a few pyknotic nuclei in some of the islet cells, one could recognize many evidences of regeneration of the acinar, centro-acinar, and islet cells. About the margin of the pancreas the lobular arrangement became quite apparent and, in fact, was exaggerated into papillomatous structures composed of a garland of acinar cells surrounding a group of centro-acinar cells and drained by one of the radicles of the pancreatic duct. In some of the larger lobules there were small groups or cords of islet cells associated with the duct or centro-acinar cells. Throughout, there appeared to be a definite increase in the number of centro-acinar cells. These were readily identified with Bowie's stain by their large, oval-shaped-pinkish staining nuclei, with single or double nucleoli, and very little if any chromatin material and clear cytoplasm. This hyperplasia of the centro-acinar cells was most apparent in the peripheral portions of the pancreas.

Our attention was then directed towards a possible anatomical explanation of the increased functional capacity of the islet system.

Hypertrophy of the islets has been observed under various conditions and has been credited with the prevention of glycosuria in such cases. Opie [13] questions the functional efficiency of these islets, as similar islets are frequently noted in association with diabetes, probably in response to functional overstrain. Such hypertrophy has been reported in diabetes by Cecil,[14] and unassociated with glycosuria in cirrhosis of the liver by Ohlmacher,[15] and about the advancing margin of malignant growths by Pearce.[16] McCallum [17]

reported two cases of diabetes in children in whom there were large islets not sharply outlined because of apparent continuation of the cell strands with the acini. The acini were also hypertrophied. Weichselbaum [18] frequently observed such changes and considered them as regeneration by the outgrowth of solid columns of cells from the ducts. It would be impossible to be certain of the nature of the cells in these large islets with the technique used by these authors.

By the use of special islet cell stains described above, the islets stood out clearly and gave one the impression of a distinct increase in number over those observed with the ordinary stains. This increase can be explained by the presence of a number of islets made up of cells with large nuclei and having a very intimate association with the acinar cells, which with the ordinary strains were very difficult to identify. With the special stains, however, more particularly Bowie's stain, the islet cells were easily identified and the characteristic staining of their granules noted.

The size, distribution, and number of islets varied considerably in different areas of the same section. Some were quite large, others small, often consisting merely of a cluster or cord of six or eight cells. As far as we could tell with the number of sections studied, the distribution of the islets throughout the whole pancreas was fairly uniform, with possibly a few more islets in the tail. What was more striking was the vast number of islets to be found in certain peripheral areas of the cross section of the pancreas as compared with the more central portions. In some peripheral areas one could identify as many as twelve to fourteen islets in a low-powered field of the microscope, whereas in the central portions we found only one islet in two and three low-powered fields. It is of course not unusual in a fibrosed pancreas, or in one showing adipose replacement, to find the islets closely packed together. In this case, however, both of these factors were absent.

We were also able to identify certain differences in the islets of the peripheral portions from those of the more central areas which lead us to believe that, broadly speaking, we were dealing with two types of islets. This distinction was not made upon characteristics of the individual cells, but rather upon their distribution, size, shape, structure, and percentage of A cells to B cells present. Neither is it suggested that they might be functionally different beyond the possible effect of a deficiency of A cells in the one group.

Throughout the central portions of the pancreas the islets as a rule were larger and contained more islet cells than one normally finds. Their cells were closely packed together and many of them showed pyknotic changes in their nuclei. They lacked the orderly structural arrangement in cords, and appeared jumbled. The outline of the islet was very irregular and islet cells could be seen extending from the main group into the surrounding acinar tissue for varying distances. No capsule, therefore, could be distinguished, although there appeared to be a normal amount of stroma supporting the individual cells. The ratio of A cells to B cells was approximately normal, the A cells representing from twenty to fifty per cent of the total number.

In the peripheral portions of the pancreas and in the centers of the finer pancreatic lobules we found islets having certain morphological changes which set them apart from those just described. As a rule they were not so large as those first described, often consisting of not more than six or eight cells. While they had no apparent capsule, they were well defined by the pronounced staining characteristics of their cells, which were also arranged in definite cords or well-defined round masses. Their cells appeared larger than the cells of the first type of islet. A small amount of connective tissue stroma supported these cells and in it were one or more blood capillaries. Their close association with the finer duct radicles and with groups of centro-acinar cells was quite obvious. In some cases they could be distinguished from centro-acinar and duct cells only by the specific staining of their granules. As an evidence of the more active growth of the islet cells we very often found them compressing the surrounding acinar cells into thin, irregular, deeply staining masses of cytoplasm. The most striking feature of these islets possibly was the almost entire absence of A cells. Occasionally we could find one or two A cells, but for the most part they were composed entirely of B cells, which were quite large with large, round or oval reticulated nuclei and which showed in their cytoplasm, with Bowie's stain, large numbers of reddish granules.

From these findings we were convinced that here we were dealing with islets which were possibly in age or time of origin different. The large central islets showing evidences of pyknotic degeneration and the lack of an orderly arrangement of their cells, and numerous A cells, we felt were older islets which existed prior to the insulin

treatment. Their increased cellularity and the spreading out of their cells into the surrounding acinar tissue suggested some attempt at regeneration.

The very numerous islets found in the peripheral areas we believe represent younger islets. This conclusion is based upon the larger size of their cells as compared with the former, their orderly arrangement in cords, absence of pyknosis, their very close association with groups of centro-acinar cells, and by the presence of practically the one type of cell, the B cell.

In view of the fact, as pointed out by Allen [12] and Homans [11] that the B cells are the antidiabetogenic cells and the ones first destroyed in severe diabetes, it is interesting to note in this case the almost complete dominance of B cells in so many of the peripheral islets as compared with those of a normal pancreas or of the central areas of this pancreas. These islets we believe are new islets which have probably developed since the commencement of insulin treatment and in functioning have been responsible in a measure for the rise in sugar tolerance. Their distribution and prominence in the peripheral portions of the pancreas suggest very strongly that they are new islets forming in the natural growth of the pancreas. The insulin treatment in relieving the strain placed upon the already functionally deficient islet system allowed the new islets to develop and assume their normal functional capacity.

Discussion by Prof. R. R. Bensley

I am very happy to be here today and to hear this case presented by Dr. Gladys Boyd. There is perhaps no problem so difficult for the pathologist to approach as that of explaining, on the basis of histological changes, why the patient improves. This case of pancreatic response to insulin treatment gives us new hopes, and increases the responsibility of the pathologist in the investigation of similar material. The interpretation of such changes as Dr. Boyd has pointed out to us is very difficult. In the absence of controls one can only imagine what the condition of the pancreas was prior to the insulin treatment, and attempt to read the process on the basis of this hypothetical condition. In Dr. Boyd's case I was more impressed with certain groups of small bulbous islands, clustered round a duct, which strongly resembled in morphology and arrangement the regenerative islands seen in experimental animals, than by

the other evidences of regeneration which she has enumerated. I have little doubt that these were actually new island units. In these cases the application of the vital-staining methods would undoubtedly render great service. I realize the difficulty of applying such methods to the study of the human pancreas, but it was accomplished successfully by Clark in several cases. The advantage of these methods is that they permit the intelligent inspection of the whole pancreas. It is the lack of such comprehensive study that is responsible for much of the confusion in interpreting the gland.

The literature of pancreatic regeneration is most unsatisfactory. A good deal is quite unreliable because of the difficulty of distinguishing between regressive and regenerative changes. The changes described by Kyrle are to my mind largely regressive. De la Roche, working under the direction of Laguesse, studied the events which followed the ligation of the pancreatic duct and found that there was considerable effort to reconstruct both acini and ducts. Some years ago I presented to the Harvey Society a description of some findings of Clark encountered in the study of similar cases of duct ligation. He found that, contrary to the usual opinion, acinous tissue did not disappear wholly until a long time after duct ligation, though the destruction of this tissue was extensive. It is fortunate that Banting supposed that the acinous tissue did disappear under these conditions, since we owe to this idea the discovery of insulin. What Banting had was probably a pancreas still containing abundant island tissue but reduced to its lowest ebb of zymogenic activity. After ligation of the duct the process was wholly destructive for a time, involving both islands and acinous tissue, but the latter more than the former. At the end of a month regenerative processes became dominant, again involving both tissues, and resulting in the formation of new acini and new islands. The newly formed acini, since they have no outlet by the duct, in turn succumb to the destructive process, but the new islands increase progressively until a maximum is reached.

In this series of experimental animals a few were encountered in which autopsy, several months after ligation of the duct, revealed a complete pancreas differing from the normal only in the fact that it was smaller in size than normal, and contained more fibrous interstitial tissue. Since this change was entirely confined to the pancreas, we interpreted these as cases in which a successful ligation

of the duct had been followed by the usual regressive changes, but in which the accidental reëstablishment of the communication of the duct with the bowel had permitted a complete regeneration of the pancreas. In one of these cases the duct had found a new opening at some distance from the stump of the old duct, which was identified at autopsy. This interpretation has been confirmed recently in my laboratory by my student Grauer, who undertook the task of reimplanting the duct into the bowel a month after a successful ligation of the duct. This reimplantation presents great difficulties and so far he had had only two successful cases out of about forty-five attempts. In every case controls of the pancreas are taken at the time of reimplantation. In one of these cases he found one month after reimplantation a complete pancreas similar in all respects to those observed by Clark. The control tissue from this animal taken at the time of reimplantation showed a typical one-month ligation picture. In the second case, examined two weeks after reimplantation of the duct, the acini were growing out into the fibrous envelope of the pancreas and covering the newly formed islets. On the basis of these experiments and observations it is my opinion that, given appropriate conditions, the capacity of the pancreas for regeneration is one hundred per cent. I believe that we shall ultimately learn how to control it. It is significant that in these observations the regenerative emphasis seems to be on the island tissue as long as the duct remains closed, but shifts to the acinous tissue as soon as communication with the bowel is reëstablished.

It is easy to see from these experiments that a pancreas obtained at autopsy in a case of diabetes may have passed through a series of kaleidoscopic changes which it would tax the imagination to figure out. For this reason great caution is necessary in the interpretation of such material.

The greatest dilemma of all in applying the insular hypothesis to the pathology of diabetes is presented by those cases in which no changes can be demonstrated in the pancreas. Various attempts have been made to explain these cases on a basis of numerical reduction of the islands, on reduction in size or in functional competency. I regret to say that I have little confidence in the results of enumeration of islands in sections. The amount of tissue which can be inspected by such methods is too infinitesimal to warrant

general conclusions. It must be remembered that we know very little about the mechanism of physiological control in the endocrine organs, and until we know more it is futile to speculate. It would be better to attempt no explanation at all of these difficult cases than to yield to the temptation to adapt the facts to the insular hypothesis. Meanwhile, such cases as the one presented by Dr. Boyd are of great importance, and will, no doubt, reward careful study by the pathologist.

Reply — Dr. Boyd

I would just like to read to you extracts from letters from Dr. F. M. Allen and Dr. Eugene Opie, giving their opinion on this case.

1. Dr. Allen: "I have the impression, first, that the islands are more numerous than should be anticipated in a child with the low tolerance which you mention before insulin treatment. Furthermore, some of the islands are large and typical, but small and irregular islands are more common than normal. They have the true capillary and trabecular framework, but the capsule is often not visible and they merge with the surrounding acinar tissue with appearances strongly suggesting 'transitions.' The special granule stain as usual excludes any true transitions between island and acinar tissue, and shows furthermore that the cytology of the islands is normal. It is altogether probable that some children at least should have the power of forming new island tissue. It has been rather surprising that this process, which must be a regular occurrence in the growing pancreas of the normal child, has been so deficient in diabetic children under diet treatment. From the combined clinical and microscopic evidence, I think you have grounds for the belief that new formation of island tissue has occurred in this case under insulin treatment. Presumably this growth has occurred by enlargement of existing islands or prolifition of ducts."

2. Dr. Opie: "I have received the second set of slides from your case of diabetic pancreas. I find in them very definite evidence of hypertrophy of the islands of Langerhans, with columns of cells extending into the acinar tissue."

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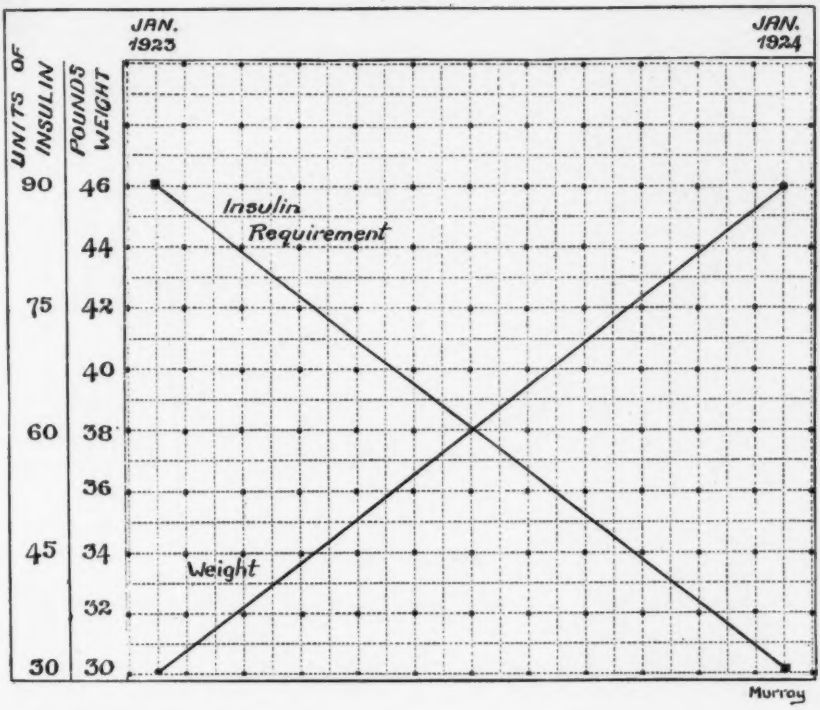
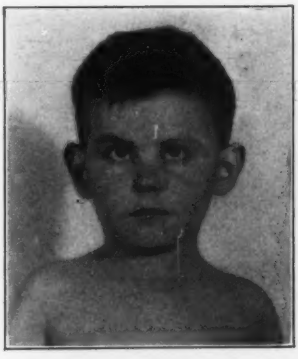
DESCRIPTION OF PLATE XXII

Chart showing decrease in insulin requirement and gain in weight of case reported.

Photographs of B. N. in January and in June, 1923.



January, 1923



June, 1923

Boyd and Robinson

Regeneration of pancreas

THE HISTOPATHOLOGY OF KALA-AZAR IN THE HAMSTER, MONKEY, AND MAN *

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The introduction of the hamster *Cricetulus griseus*, M.-Edw. as an experimental animal in the study of kala-azar in North China has furnished an abundance of material for histological research. This study has not only demonstrated clearly the pathological picture already described by many investigators in other animals and in man, but it has brought out some fundamental points in the pathology which have not been emphasized heretofore. I venture, therefore, to present our microscopic findings in the hamster in considerable detail and to compare them with our findings in the monkey and in man.

HISTORICAL

The first careful description of the histopathology of kala-azar was made by Christophers.[1] His accurate descriptions and interpretation of the pathological process have not been greatly added to by subsequent writers. He saw that the tissue changes which were due directly to the Leishman-donovan body involved mainly the presence and multiplication of the parasite in a certain group of cells represented by the endothelial cells of the liver and spleen capillaries and by the large mononuclear phagocytes of the blood and tissues. He recognized also the very important fact that Leishman-donovan bodies apparently proliferated within the cytoplasm of these phagocytic cells and were not destroyed by them, but ultimately caused their bursting or disintegration, followed by the liberation of the parasites into the tissues or blood stream. Christophers also appreciated the relationship between the pathological process in kala-azar and that in cutaneous leishmaniasis, which had been described by Wright [2] a short time before.

Since the appearance of these papers a large number of articles have appeared dealing with the pathology of the cutaneous and

* Received for publication December 21, 1924.

visceral leishmaniases. The British workers in India, and the Italian and French in the Mediterranean field have contributed most to the literature. More recently the South American cutaneous lesions have been carefully studied. Jemma and Di Cristina [3] describe the phagocytic cells as they appear in infantile kala-azar in Italy, designating them "blackberry-shaped cells" when they are distended with Leishman-donovan bodies. They discuss the distribution of the parasites and of the phagocytic cells and emphasize the relation of the latter to the vascular endothelium.

With the discovery of spontaneous leishmaniasis in dogs in Italy, and with the successful transmission of kala-azar to monkeys, dogs, and mice, an abundance of histological material became available. The best summary of the findings in experimental animals and in human cases is that of Laveran.[4] He shows that, in practically all positive cases, the liver, spleen, and bone marrow are involved in the pathological process, and that the intensity and duration of the infection determine to what extent other organs are involved. After the blood-forming organs, the lymph nodes and lymphatic tissue in other organs next become involved, then the organs whose loose areolar tissue normally contains many small blood vessels and wandering cells. Thus the intestines, testes, lungs, adrenals, pancreas, skin, kidneys, and meninges are likely to become involved in about the order named. The thymus has also been found to contain organisms. In all the visceral forms of the disease, however, including Indian, Mediterranean, canine, and experimental, the essential lesion appears to be the same. Emphasis has been laid by all authors upon the parasitizing of endothelial cells and the accumulation of these cells in favorable locations, particularly in the organs of the hematopoietic system.

Two recent papers on the histopathology of kala-azar deserve special mention. The first is that of Perry,[5] who describes his findings in the jejunum of two human cases of Indian kala-azar. The stroma of the mucosa was distended by extensive proliferation of the endothelial cells lining the lymph channels, and these cells were filled with Leishman-donovan bodies. The epithelium covering the villi was absent, but it persisted in the bases of the crypts between the villi. Although this, we believe, must have been due to post mortem change or to trauma in washing the tissues at necropsy, the findings emphasize the massive accumulation of parasitized cells

in this location. Interpreting the loss of epithelium in his sections as part of the pathological process, Perry emphasizes the possibility of the elimination of the parasite in viable form by way of the intestinal tract.

The second recent paper of interest is that of Shortt,[6] who describes the histological findings in two monkeys infected by intraperitoneal inoculation of liver and spleen emulsion from a case of Indian kala-azar. Shortt emphasizes the localization of the parasites in the endothelial cells of blood capillaries in the liver, spleen, and bone marrow, and in the interstitial cells of Leydig in the testis. In connection with the last of these findings, we shall describe later in this paper the findings in a human testis in which we are able to differentiate between the cells of Leydig and those containing parasites.

The invasion of the parenchymatous cells of the liver and suprarenal gland by Leishman-donovan bodies has been described by a few authors. Havet,[7] working in Laveran's laboratory, described the tissues of a dog infected experimentally by material from another naturally infected dog. In the liver he found the parenchymatous cells of the organ more heavily invaded by parasites than the capillary endothelium. Some of the liver cells also contained polymorphonuclear leucocytes. Such extensive invasion of the liver cells has not been reported by any other writer, but several have reported the occasional parasitization of liver cells, and we are able to corroborate this finding, as will be seen later.

Jemma and Di Cristina[3] show a colored drawing of a glandular cell of the suprarenal cortex invaded by Leishmania. We also found these cells parasitized in our most heavily infected hamster.

Nowhere in the available literature have we found a description of such focal lesions in the organs of either human cases or animals, as appear in the tissues which we are to describe in this paper. The appearance of a local proliferation of phagocytic cells forming masses of tissue with an epithelium-like appearance in many of the organs involved has been so striking as to command special attention, and the fact that the cells making up these masses often contain very few or no Leishman-donovan bodies indicates, we believe, a specific tissue response to the invading organism, which has not hitherto been emphasized.

Although no previous descriptions of the histopathology of kala-

azar in North China have been published, we have no grounds for believing that the disease or its pathology is different from that in India. The literature, furthermore, emphasizes the similarity in the pathology of the Indian and Mediterranean forms of the disease, and the similarity of findings in human cases and in experimentally and naturally infected animals.

Material for Study

The material used in the present study includes tissues from nineteen hamsters, two monkeys, and two human cases. Tissues from the hamsters were kindly provided by Dr. C. W. Young and Dr. H. J. Smyly of this department. The tissues were taken immediately after death, the animal either being sacrificed for the purpose or having been killed accidentally by liver puncture. The two monkeys were killed when moribund. The human tissues were secured one-half an hour and eighteen hours, respectively, after death. All tissues except those used for fat stain were fixed in Zenker's solution, were imbedded in paraffin, and cut at about five micra. Harris's hematoxylin counterstained with eosin was found to give a better combined tissue and parasite picture than the eosin-azur of Giemsa, and was therefore used throughout the study. Sections to show fat were fixed in formalin, cut by a freezing microtome, stained with Scharlach-R and counterstained with Harris' hematoxylin. For demonstration of the fibrous reticulum of the spleen, Mallory's aniline blue stain with carmine counterstain was used on Zenker-fixed paraffin sections.

Kala-Azar in the Hamster

The hamster, *Cricetulus griseus*, M.-Edw., is a grayish-brown field mouse with a white throat and belly (Fig. 1). The adult measures about 8 cm. in length and weighs about 30 grams, the male being somewhat larger than the female. The male has very large and prominent testes which cause the scrotum to protude backward from the body at the base of the short tail. These rodents inhabit the dry fields about Peking and are caught by the farmers in large numbers. They are easily kept in captivity, but do not breed unless furnished with a tunnel of earth in which to nest. They can be readily infected with kala-azar by the intraperitoneal inoculation of small amounts of an emulsion of infected human or animal tissue.

Infection by the use of cultures of *Leishmania donovani* has not yet been attempted. The two unique features of the infection in these animals are, first, the speed and ease with which a successful inoculation can be demonstrated, and, second, the increasing intensity of the infection in an inoculated animal over a long period of time with only slight jeopardy to the health of the animal. Of the two hamsters in our series which were longest infected, one was killed while in apparently good health on the 455th day after infection, and the other on the 351st day because it developed an abscess in one of its cheek sacs. Our 90-day hamster was definitely ill when killed. Some of the other hamsters infected in our laboratories have died a natural death probably as a direct result of the kala-azar infection, but their tissues could not be used because of rapid putrefaction. Young, Smyly, and Brown [8] have shown that smears of the spleen of hamsters reveal Leishman-donovan bodies on the third day after infection, and of the liver and bone marrow on the fourth day. Liver puncture, which can usually be done without killing the animal, reveals organisms with almost as great accuracy.

The macroscopic examination at necropsy of hamsters infected with kala-azar usually reveals nothing pathological except an enlarged spleen. In infections of one hundred or more days' duration this reaches enormous proportions. The organ may extend across the mid-line in the lower abdomen, reaching the right iliac fossa. The normal weight of the hamster's spleen is 0.05 to 0.1 gm. (0.15 to 0.3 per cent of the body weight). In hamster S. 22, infected 455 days, the spleen weighed 1.0 gm. or 3.1 per cent of the body weight. The liver ordinarily shows no macroscopic pathology, but in this same hamster it was greatly enlarged, extending nearly half way from the costal margin to the pubis, and was pale brown in color with many irregular light-red hemorrhagic areas on its surface. Other organs in this hamster might have shown macroscopic lesions had the organs been larger. This will be evident from the microscopic findings.

Microscopic Findings in the Hamster

A. *The Fundamental Tissue Reaction.* Before describing in detail the microscopic findings in the various organs, we wish to lay emphasis upon the specific tissue reaction of the mammalian body to the invading organism. This occurs in all tissues where a more or

less loose connective tissue stroma exists in which cells of the specific reactive type normally occur. The cell involved is the large mononuclear phagocyte of mammalian tissues, which has been variously designated as clasmatocyte (Ranvier), histiocyte (Aschoff), endothelial leucocyte (Mallory), macrophage (Metchnikoff).*

This cell, which we shall hereafter designate as the clasmatocyte, exists primarily in the reticulum of the spleen, lymphatic tissue, and bone marrow, but is found also in small numbers in the reticular connective tissue of the intestinal mucosa, the testis, and the other glandular organs. The subcutaneous and other connective tissues of the body may also contain it. In the liver the endothelial cells lining the intralobular blood capillaries (the so-called Kupffer cells) seem to belong to this group. This relationship is made simple if we assume, as seems probable, that the clasmatocyte is originally derived from the endothelium of the blood and lymph vessels.

In hamsters heavily infected with kala-azar, we find, in the organs mentioned above, islands of clasmatocytes forming solid masses of tissue (Figs. 28, 29, and 30). Wherever the structure will allow it, the cell masses occupy the reticular connective tissue. They may impinge directly on blood or lymph vessels, and may even project as minute polyps into the lumina of such vessels. The cells are closely set together as though forming a definite structure, and frequently give the appearance of a mass of pavement epithelium. This may be designated "clasmatocyte tissue" in the same sense as we speak of "lymphoid tissue."

The individual cells vary in size and shape according to their location, but always have a considerable amount of cytoplasm which is finely granular and stains fairly deeply with eosin. At the periphery of the cell masses, long processes of cytoplasm may project out into the loose connective tissue. The nucleus, usually single but occasionally multiple, is oval, reniform, or horse-shoe shaped. It is vesicular, contains one or two spherical nucleoli paracentrally located and three or four other chromatin masses which are usually flattened against the nuclear membrane. There is a very fine chromatin network which radiates outward from the chromatin masses and from

* The recent work of Cunningham, Sabin, and Doan,[9] separating the phagocytic cells of the spleen into two distinct groups, raises the question whether both these types of cell[3] are equally phagocytic for *Leishmania donovani*. We hope to investigate this point in the near future.

the nucleolus where it is densest. By these features this cell may readily be distinguished from glandular epithelial cells, from the interstitial cells of Leydig in the testis, from lymphocytes and plasma cells, and from smooth muscle cells. It is difficult to distinguish in nuclear structure from reticular connective tissue cells and from swollen endothelial cells. Its relationship to both these may be very close.

In the solid masses of these cells one sometimes finds scattered cells of other types; lymphocytes and plasma cells are most common, occasionally polymorphonuclear leucocytes and red blood cells are found, and of course, reticular connective tissue cells. Rarely lymphocytes or red blood cells are found within the cytoplasm of the phagocytes. In definite patches of clasmatocyte tissue the most striking thing is the slight degree to which the cells are parasitized. Most of them contain a few or a moderate number of parasites, but some cells, particularly in the centers of the cell masses, contained none. At the periphery some may be crowded with parasites. Occasionally a few cells, even when not heavily parasitized, have nuclei of a degenerated appearance, either staining diffusely blue, or taking a faint reddish tinge. When heavily parasitized, the nucleus of the cell may be shrunken or missing, and the cytoplasm may contain vacuoles which often seem to be occupied by the parasites. In very heavily infected tissues small holes containing a few Leishman-donovan bodies may be the only remnant of cells which have burst as a result of the proliferation of the parasites.

We have not seen definite mitotic figures among these masses of cells, although they do occur in some organs in cells which seem to be clasmatocytes. This lack of mitotic figures is strange, for it is difficult to explain the local accumulation of these cells except that they multiply *in situ*. Furthermore, the local multiplication seems to precede extensive parasitization for we have found the cell masses in the spleen, liver, and lymph nodes of mice infected only eleven days. Such an early development of groups of specialized cells and their mild degree of parasitization emphasizes the fact that this is a proliferative tissue response to a definite stimulus. The picture is clearest in animals in which the infection has reached a moderate degree. In those which are most heavily infected the very extensive proliferation of clasmatocytes and their intense parasitization hide somewhat the typical picture.

B. *The Characteristics of Leishmania donovani in tissues.* The typical Leishman-donovan body as seen in histological sections stained with hematoxylin and eosin is an oval body measuring about two by three micra. It has a definite pink-staining outline, but the cytoplasm is usually very faintly pink or colorless. Near one end is the spherical macro-nucleus about one micron in diameter, which usually has an eccentric clear circular area within it. The minute rod-shaped micro-nucleus points readily away from near the macro-nucleus toward the other end of the organism. The cell outline of the organism often cannot be seen, particularly if the cytoplasm of the parasitized cell stains deeply. Frequently the micro-nucleus is also invisible, perhaps because it is hidden by the macro-nucleus. Obviously it is impossible to distinguish with certainty a single *Leishmania* in a tissue section unless it is characteristic in appearance, and this may depend merely on its position with reference to the observer's eye. This explains why it has been possible for Young, Smyly, and Brown to find *Leishmania* in smears made from the spleen, liver, and bone marrow of hamsters on the third or fourth day after infection, whereas, in the same series of animals, our microscopic sections failed to reveal them until the sixth day. This is also probably the reason why sections from human necropsies sometimes fail to show organisms, while smears from the same organs made at necropsy do show them.

C. *The development of the pathological process in the organs involved. — Liver.* Leishman-donovan bodies are first found in the six-day hamster, where a few single Kupffer cells contain small numbers of parasites. In the nine-day animal a few small groups of proliferated endothelial cells project into the lumina of capillaries. By the eleventh day, there are many small islands of proliferated endothelial cells in some of which one or more cells contain parasites. In the portal spaces are a few parasitized clasmatocytes. The proliferation and parasitization steadily increased in intensity. *In the 115-day animal one liver cell was seen which contained parasites in its cytoplasm. 125-day hamster:* Islands of parasitized endothelial cells still larger and more numerous. Liver cells about them compressed slightly. A few liver cells contain parasites, sometimes numerous, and have cytoplasm slightly fragmented. Some of the Kupffer cells from capillaries have proliferated so as to project into the lumina of central lobular veins (Fig. 2). In some of these veins many parasitized clasmatocytes are present free among the blood cells (Fig. 3). This is in contrast to the portal veins, where no parasitized cells are found in the lumina. In the portal spaces several layers of clasmatocytes surround the veins, ducts, and arteries (Fig. 4). *351-day hamster:* The periportal proliferation of clasmatocytes is less marked, but otherwise the process is much farther advanced. Many liver cells are parasitized and show profound changes (Fig. 6). Large masses of endothelial cells

occlude many of the capillaries and are crowded with parasites; many contain large vacuoles, and a few are in fragments (Fig. 5). Many of their nuclei are shrunken. A few mitoses are seen among these cells (Fig. 7). The portal spaces show a marked increase of lymphocytes and plasma cells, besides the numerous parasitized clasmatocytes. A few small groups of plasma cells are present in the intralobular capillaries. Clasmatocytes sometimes abut directly on the lumen of the portal vein and a few are free in the lumen. In the central veins are masses of parasitized cells protruding into the lumen, but their continuity with the endothelium of the vein is not distinct (Fig. 8). An occasional flat endothelial cell lining a central vein contains a few parasites. *455-day hamster:* Profound changes have taken place in the liver tissue (Fig. 9). The lobular architecture of the organ is indistinct. The tissue is very friable. The liver cells are nearly all smaller than normal and some are shrunken or compressed into thin strands of tissue, between widely dilated capillaries (Fig. 10). They show all degrees of degeneration and parasitization. From ten to one hundred per cent of the liver cells in various fields are parasitized. In the areas where most degeneration is present some liver cells contain large fat globules. In some areas practically no liver cells remain, the loose tissue being made up almost entirely of heavily parasitized endothelial cells which float like balloons in the wide blood spaces. These cells are larger than in any previous hamster, sometimes measuring fifty micra in the smaller diameter, and containing hundreds of Leishmaniae in a single section. They are not grouped in very large masses, fifteen cells in one mass being about the maximum for one section. Many have no nuclei and others are vacuolated or fragmented. The portal spaces contain very few cells and the interlobular bile ducts have shrunken epithelium and practically no lumina. The central veins are not remarkable except that some of their flat endothelial cells contain parasites.

Spleen. The nine-day hamster was the first in which parasitized clasmatocytes were found. In later animals one notes first a larger number of single clasmatocytes in the pulp (11-day hamster), then a number of islands of clasmatocytes, most of which contained parasitized cells (15-day hamster). In the 90-day animal much of the pulp is replaced by clasmatocyte tissue, and some of the lymph follicles are invaded by it. *125-day hamster:* The picture is similar but more advanced, the pulp being more completely occupied by clasmatocytes, which are nearly all heavily parasitized, greatly swollen and often vacuolated (Fig. 11). Some have become disintegrated, leaving open spaces containing a few parasites. Many contain red blood cells as well as parasites. The blood capillaries and sinuses are somewhat compressed by the surrounding cells. The lymph follicles are more extensively invaded by clasmatocytes; some are almost completely replaced by clasmatocyte tissue (Fig. 12). *455-day hamster:* Huge heavily parasitized clasmatocytes dominate the picture. They occupy practically the entire pulp, and partly replace the lymph follicles (Figs. 13 and 14). The blood is mostly confined to definite vessels of narrow calibre. Most of these vessels have only a single layer of flat cells separating the lumina from the clasmatocytes, and even they are sometimes not visible (Fig. 15). Some vessels contain parasitized cells. Only thin trabeculae of fibrous tissue are present and seem to be compressed by the surrounding cells. The arteries of the lymph follicles often cannot be found. No mitotic figures are seen either in the pulp or in the splenic corpuscles where they are usually numerous.

Lymph Nodes. The first change seen was (15-day hamster) proliferation of

clasmatocytes just outside the mesial wall of the peripheral lymph sinus and beginning to invade the lymph follicles. A few of these cells contained Leishman-donovan bodies. This invasion of the follicular tissue steadily advanced, and the follicular architecture of the nodes was soon lost. The clasmatocyte tissue seemed to extend inward from the region of peripheral lymph sinus (Fig. 16). Smaller islands of this tissue were also present in the medullary portion of the node. *The degree of parasitization of any one group of cells seems to be in inverse proportion to the number of cells in the group.* In the 125-day hamster a considerable number of the parasitized clasmatocytes have several nuclei, sometimes grouped in the center, sometimes forming a ring (Fig. 17). This may be one method of division of these cells into a tissue-like group. *455-day hamster:* About half the lymphoid tissue is replaced by clasmatocyte tissue, and besides this many single clasmatocytes are present among the remaining lymphocytes. Nearly all the clasmatocytes are heavily parasitized, many are vacuolated or have burst. Lymph sinuses are dilated and contain many parasitized cells.

Bone Marrow. We failed to find Leishman-donovan bodies in sections of bone marrow until the twenty-fourth day after infection. This is in contrast to the finding of organisms in bone marrow smears in similar hamsters by Young, Smyly, and Brown on the fourth day after infection. It is difficult to obtain perfect sections of bones without decalcification, and that process frequently makes good staining impossible. This, together with the difficulty of recognizing single Leishman-donovan bodies in sections, probably accounts for the discrepancy. The bone marrow of the shafts of the femur and humerus is normally hematopoietic in the hamster, but very few clasmatocytes are found there normally. The femur, humerus, and vertebrae were the bones used in our study. *24-day hamster:* Active hematopoietic tissue lies between numerous fat cells. A few single clasmatocytes are present, crowded with Leishmaniae. *35-day hamster:* Throughout the marrow are many heavily parasitized clasmatocytes. They occur both singly and in islands of as many as twenty cells each. In the islands the cells fit close together like a pavement. *125-day hamster:* About one-third of the marrow is occupied by clasmatocytes, most of which are heavily parasitized, resulting in huge globular cells (Fig. 18). These cells often contain remains of other cells also, either red blood cells, lymphocytes, or granular leucocytes. *455-day hamster:* The clasmatocyte tissue comprises about one-half of the marrow (Fig. 19). The parasitized cells are huge and lie closely packed together. The hematopoietic cells lie in small compact groups between the masses of clasmatocytes. Erythropoiesis seems to be greatly reduced, and the blood vessels are apparently compressed.

Adrenal. Parasitized clasmatocytes were first seen in the 90-day hamster, in the interstitial tissue of the cortex and medulla. These increase in number and become grouped into small masses in later animals. In the 455-day hamster a few of the parenchymatous cells of the cortex contain Leishman-donovan bodies (Fig. 20). The parasitized cells show shrinking or fragmentation of the nuclei and fragmentation of the cytoplasm.

Outside of the five organs so far described, the localization of parasitized clasmatocytes is limited, except for rare instances, to the connective tissue of the body. The most favorable sites for this localization are the regions where loose areolar tissue is associated with good vascularity. Both of these factors probably operate to favor this localization as illustrated on the one hand by fat tissue, which is loose but not very vascular, and on the other hand by the

interstitial tissue of the pancreas and salivary glands, which is not loose but is very vascular. Where both conditions are present, as in the stroma of the intestinal mucosa, the pathological picture becomes very intense. In the kidney, however, the interstitial tissue contains parasitized cells only in the most intense general infections, and in the brain not at all. In order to conserve space the further findings will be described only from the 455-day hamster.

Fat Tissue. Wherever fat appears, single clasmatocytes, heavily parasitized, are scattered through it (Fig. 21). Very seldom are these accumulated into cell groups.

Gastro-Intestinal Tract. Stomach — heavily parasitized clasmatocytes are present in large numbers in the submucosa. They are also scattered singly throughout the stroma of the mucosa. *Duodenum* — the villi are mostly bulbous in shape due to the massing in the stroma of huge parasitized clasmatocytes (Fig. 22). These cells are packed closely together and replace the normal cellular content of this tissue. This is so especially toward the tips of villi, their bases often being relatively normal. The epithelium covering the villi is usually intact and no Leishmaniae are seen in the epithelial cells. The epithelium is separated from its basement membrane by a space filled with granular, pink staining material. This has been seen also in sections from other hamsters where no parasitized cells were present, and is probably a normal condition. Numerous parasitized cells are present also in the submucosa, except where Brünner's glands occur. *Jejunum* — much the same picture, but less intense. The tips of some villi show autolysis. *Ileum* — still less intense, perhaps because villi are small. Where lymph nodules occur they are partly replaced by parasitized "clasmatocyte tissue." Epithelium everywhere intact. *Cecum and Colon* — much less intense. Only single parasitized cells are present, scattered thickly through the mucosa and submucosa. Epithelium intact. In all sections from the gastro-intestinal tract, there are many single parasitized cells in the connective tissue septa of the muscularis and in the serosa.

Lungs. Both in the interlobular tissue and in alveolar walls parasitized cells are numerous. In the alveolar walls their relation to capillaries and epithelium is impossible to demonstrate. There is no apparent proliferation of capillary endothelium.

Heart. Throughout the myocardium and the sub-endocardial tissue heavily parasitized clasmatocytes are present in the interstitial tissue. Several groups of these cells are apparently associated with lymph vessels. Their relation to blood vessels is not so close. No parasitized cells are found lining the epi- or endocardium. The epicardial fat is heavily infiltrated with parasitized cells single and in groups. Muscle cells normal.

Pancreas and Salivary Glands. The interstitial tissue in these glands contains many single parasitized clasmatocytes.

Kidney. The kidney tissue in all previous hamsters was normal, but in this one a remarkable pathological condition is present. Throughout the cortex the proximal convoluted tubules are in all stages of degeneration, some normal, some represented only by a wrinkled basement membrane. Although there is very little formation of hyaline droplets in the tubular epithelium, the cells show all degrees of fragmentation and atrophy, the nuclei being the last portion of the cells to disappear. Many tubules contain hyaline casts. In proportion to the degree of tubular degeneration the interstitial tissue is infiltrated with clasmatocytes heavily laden with Leishmaniae. Several large and small areas

of this atrophy and infiltration are present and irregular strands of such tissue run through the cortex. The glomeruli are normal except for occasional distention of Bowman's capsule and for the presence of parasitized cells. These average one to a glomerulus in a section. They always appear to be filling the lumen of a capillary, but whether the cells are capillary endothelial cells or clasmatocytes plugging the vessels as emboli cannot be determined. In the medulla many collecting tubules are dilated and others are plugged with casts. In striking contrast to the cortex, the medulla contains no parasitized cells in its interstitial tissue.

Bladder, Testis, Epididymis, Seminal Vesicles. A few parasitized clasmatocytes are present in the connective tissue.

Prostate. Beneath the simple columnar epithelium is a densely cellular connective tissue. The invasion of this tissue by parasitized clasmatocytes approaches that of the intestinal mucosa in intensity (Fig. 23).

Brain and Meninges. Neither in the brain tissue itself nor in the walls of intracerebral blood vessels are any parasitized cells found. In the meninges and choroid plexus, however, they are rather numerous, usually in the loose tissue just outside blood vessels, but occasionally in the intimal cells of capillaries.

Eye. Parasitized cells found only in the loose outer layers of the posterior sclera where it joins the orbital connective tissue.

Skin and Cheek Sac. Beneath the epithelium in the loose areolar tissue parasitized clasmatocytes are very numerous.

Monkeys

Two female monkeys, *Pithecus irus* (Syn. Macacus irus), were inoculated intraperitoneally with a suspension of liver and spleen from hamsters infected with kala-azar. The material for inoculation was ground up in a Rosenow tissue macerator and suspended in 10 c.c. of Locke's solution. Liver puncture first positive for *Leishmania donovani* on the 10th and 7th days respectively after inoculation. Both monkeys showed gradual progressive emaciation and lassitude, huddling up in one corner of cage. They became moribund on 45th and 48th days respectively and were killed by ether on those days.

Monkey 1. Necropsy. Very little subcutaneous fat. Spleen weighed 23 grams and was much enlarged, dark red in color, firm, not friable. Cut surface dark red, lymph follicles scarcely visible. Liver also much enlarged, weight 92 grams, otherwise grossly normal. Stomach contained an ulcer near pylorus 1 cm. in diameter, with raised margin and depressed center. Smear from this showed no parasites of any kind. Another ulcer was found in the rectum, probably due to taking temperature. Lymph nodes all slightly enlarged. Bone marrow of shaft of femur red and gelatinous. Other organs macroscopically normal.

Microscopic Sections.—Liver. Intralobular blood capillaries irregularly distended by intense proliferation of endothelial cells which often occlude the lumen entirely. In the endothelial cells are varying numbers of Leishmaniae, but rarely more than ten in a single cell in one section. Some cells contain none although they form part of a mass of endothelial cells. Many of the parasitized cells are vacuolated and Scharlach-R stain shows them to be heavily laden with fat. Mitotic figures are common in the masses of endothelial cells. Many nucleated red blood cells in the capillaries. Liver cells appear normal. None is

seen to contain Leishmaniae. The proliferation of endothelial cells extends on the one hand into the portal veins, the lumina of many of which are nearly occluded, and on the other hand into the central efferent veins, in which masses of parasitized endothelial cells lie in more or less intimate contact with the vessel wall. Blood-vessels contain very little blood. Portal spaces show little or no cellular infiltration and contain very few parasitized cells.

Spleen. Contrary to expectations only a very few Leishman-donovan bodies are found. Lymph follicles small and often partly replaced either by loose reticular tissue containing open spaces partly filled with blood, or by degenerating cellular material. This consists mostly of cells with large pale vesicular nuclei much like clasmatocytes, but the nuclei are often shrunken and the cytoplasm is indefinite in outline. There is considerable nuclear débris. Pulp consists mainly of this same semi-degenerated tissue, in which considerable blood is present. Considerable proliferation of fibroblasts, with an occasional mitosis. An occasional faintly-staining Leishman-donovan body is seen in the cytoplasm of a clasmatocyte, but the tissue pathology is far out of proportion to the apparent number of parasites.

Lymph Nodes. The picture varies in different nodes. In some the lymph nodules are well preserved, usually without germinal centers, and the invasion of clasmatocytes into lymph nodules is slight. In these nodes, however, Leishman-donovan bodies are present in most of the clasmatocytes, both in the reticulum of the node and in sinuses. In other nodes the lymphoid tissue is largely replaced by masses of cells with the characteristics of clasmatocytes, but there is much cellular fragmentation with free chromatin masses. A few Leishman-donovan bodies are seen in the clasmatocytes of these degenerated areas, but they usually stain faintly, and the impression received is that many more are present which have lost their staining power. Normal lymphoid tissue and degenerated regions may appear in the same section.

Bone Marrow. Very cellular, showing all the normal elements of active marrow, and in addition many clasmatocytes. These occur both singly and in groups. Most of the single ones are heavily parasitized. Where groups of cells occur, however, the cytoplasm of individual cells stains deeply pink, and only a few of the cells contain Leishmaniae. Most of these groups of cells have at the center a circular open space about 50 micra in diameter, about which a few flattened nuclei are usually present. Such spaces occur only in connection with these groups of clasmatocytes.

Adrenal. In the interstitial tissue between the glandular cells of the cortex are a few clasmatocytes containing Leishman-donovan bodies. The cortical cells contain practically no lipoid material. Otherwise the organ is normal.

Gastro-Intestinal Tract. In the stroma of the intestinal mucosa are varying numbers of parasitized clasmatocytes, most numerous in the jejunum. In the appendix, they are numerous about the periphery of a lymph follicle and in a lymph vessel leading away from this follicle (Fig. 24). Ulcers of stomach and rectum are not remarkable.

Lungs. The picture of endothelial proliferation with Leishman-donovan bodies is very similar to that in the liver. The lumina of the small arteries, veins, and capillaries are often practically occluded by masses of endothelial cells attached to the vessel wall (Fig. 25). Some of these cells contain Leishmaniae. The cells are often degenerated, containing large vacuoles. No cells of any kind are present in the alveoli, nor are any Leishman-donovan bodies

seen in cells lining the alveoli. There is marked congestion of those capillaries which are not occluded. In one section considerable interstitial hemorrhage is present. In the interlobular septa, cells heavily pigmented with carbon are present, but no Leishman-donovan bodies are found in those cells.

Heart. There are several patches in the myocardium where the muscle cells are swollen and hyalinized. There is one small hemorrhage. No Leishman-donovan bodies are found.

Kidney. A few swollen endothelial cells containing Leishmaniae are found in the veins and in capillaries of the glomeruli. Otherwise the organ is normal.

Brain. A few parasitized cells are found in the lumina of capillaries in the meninges and choroid plexus. Sections otherwise normal.

Sections of pancreas, salivary glands, urinary bladder, uterus, skin, and spinal cord all normal. The tongue and striated muscle in the neck show several colonies of sarcosporidia lying in or between muscle cells.

Monkey 2. Necropsy. Spleen and liver not so much enlarged as in Monkey 1, weighing 13 and 78 grams respectively.

Microscopic Sections. The picture of endothelial proliferation in the liver and lungs is even more marked than in Monkey 1 (Figs. 26 and 27). The other organs show in general the same histo-pathology. Leishman-donovan bodies are not well preserved in any organs except the colon and appendix. The impression is conveyed that extensive destruction of the parasites had taken place.

Human Cases

Case 1. P. U. M. C. Hospital No. 3853. Mrs. C. Y. T. G., a Chinese housewife, 38 years old, had had symptoms referable to kala-azar for nine months before death. She had had tartar emetic treatment in another hospital but had refused to continue it. She died of broncho-pneumonia on December 18, 1922.

Necropsy, by Dr. C. Bartlett (45 minutes post-mortem). Anatomical Diagnosis — Broncho-pneumonia, pleural adhesions, splenomegaly (485 grams) multiple old splenic infarcts, fatty liver, old and recent infarcts of right kidney, peritoneal and pericardial effusions.

Microscopic Sections. (H.E.M.) *Liver.* Extreme fat infiltration, most intense about the central veins. The few liver cells which are not laden with fat contain bile pigment, and a few are parasitized with *Leishmania.* In the blood capillaries the Kupffer cells are swollen and heavily parasitized, but there is very little proliferation of these cells into masses as in the hamsters and monkeys. Some of the parasitized cells also contain red blood cells or lymphocytes. In the walls and lumina of both portal and efferent veins are parasitized clasmatocytes. The portal spaces, however, show neither thickening nor cellular infiltration.

Spleen. Throughout the pulp, both in the reticulum and in the blood sinuses are myriads of heavily parasitized clasmatocytes. The infiltration is as heavy as in the 351-day hamster, but the parasitized cells are less swollen and fragmented, and there is less tendency to massing of parasitized cells into definite tissue. They encroach very little on the lymph follicles, which are about normal in size, although they have no germinal centers and often contain masses of hyaline material suggestive of amyloid. The pulp is greatly congested with blood, and also contains many lymphocytes and plasma cells. There is perhaps a slight increase of the fibrous reticulum.

Lymph Nodes. In some nodes the reticulum and sinuses contain diffusely

distributed clasmatocytes, most of which are heavily parasitized. In other nodes a large part of the reticulum is filled with definite "clasmatocyte tissue" as in the hamsters. When this occurs the clasmatocytes contain few or no Leishman-donovan bodies and their cytoplasm stains a deep pink. Carbon pigment, red blood cells and Leishman-donovan bodies are found together in some clasmatocytes. The lymph follicles are small and have no germinal centers.

Adrenal. Deep in the cortex are a few collections of lymphocytes, among which are a few parasitized clasmatocytes. Otherwise normal.

Lungs. Only an occasional parasitized clasmatocyte is seen in the alveolar walls. No endothelial proliferation is evident, as in the monkeys. There is a purulent bronchitis and in places a peri-bronchial pneumonia. There are also hemorrhages into groups of alveoli and in the patches of pneumonia. In nearly all the alveolar cavities are clasmatocytes, many of which contain carbon, but no Leishman-donovan bodies are seen in them.

Kidneys. A few parasitized clasmatocytes are found plugging glomerular capillaries.

Sections of heart, aorta, pancreas, gall bladder, urinary bladder, and ovary essentially normal. No specimens of bone marrow or gastro-intestinal tract were preserved.

CASE 2. P. U. M. C. Hospital No. 1757. H. S. T. Chinese man, student, 23 years old, was discovered to have kala-azar eighteen months before death. He failed to continue treatments, and died of a pneumococcus type III pneumonia on March 6, 1924.

Necropsy, by Dr. Y. K. Wang (18 hours post-mortem).

Anatomical Diagnosis. Lobar pneumonia, right; suppurative pluritis, left; suppurative pericarditis; bacteriemia, pneumococcus type III; splenomegaly (1,355 grams), enlargement of liver (12.5 cm. below costal margin); enlargement of mesenteric and cervical lymph nodes; hyperplasia of bone marrow.

Microscopic Sections. (H.E.M.) *Liver.* In the blood capillaries are many Kupffer cells heavily parasitized with *Leismania.* These are usually single, but a few groups of four or five cells are seen. In the portal spaces are many parasitized cells, often abutting directly upon lymph and blood vessels.

Spleen. Almost the entire tissue is composed of a reticulum containing "clasmatocyte tissue." Most of the clasmatocytes contain a few Leishmaniae; none is very heavily parasitized. The blood sinuses in general are widened and their walls thickened, but there is practically no proliferation of endothelial cells. There is a definite increase of the reticular fibrous tissue of the organ.

Lymph Nodes. The mesenteric nodes all show marked autolysis but in the loose reticulum which remains are many disintegrating cells heavily laden with Leishmaniae. The bronchial and aortic nodes show a loss of follicular architecture, although lymphocytes are still the predominating cells. There are also many clasmatocytes, both in the reticulum and in sinuses but only an occasional *Leishmania* is seen in them. There is no definite "clasmatocyte tissue." Several of the nodes contain areas of tuberculous caseation with giant cells.

Bone Marrow. (Rib.) This is perhaps better than the bone marrow of the femur for studying the specific reaction of kala-azar, since it is normally active marrow, and is therefore not complicated by the picture of hematopoiesis which is stimulated in the femur by this disease. This marrow contains no fat, and approximately eighty per cent of the space is occupied by clasmatocytes, packed

closely together into definite "clasmatocyte tissue." The picture is more striking than that found in any of the hamsters. Most of these cells contain a few Leishman-donovan bodies, some do not show them in the sections studied, none is very heavily parasitized. The normal hematopoiesis is almost entirely absent, especially the erythropoiesis. Most of the cells aside from the clasmatocytes seem to be lymphocytes. There are a few myelocytes and megakaryocytes. The vascularity of the marrow is greatly reduced.

Adrenal. In the medulla are a few collections of lymphocytes among which an occasional parasitized clasmatocyte occurs. In the fat tissue outside the organ is a focus of about one hundred cells, lymphocytes, plasma cells, and parasitized clasmatocytes.

Gastro-Intestinal Tract. There is marked post-mortem autolysis of the mucosa in most of the sections studied. *Stomach* — A few heavily parasitized clasmatocytes in superficial portion of stroma of mucosa. *Jejunum* — superficial portions of villi crowded with parasitized cells. *Ileum, cecum, appendix,* and *colon* — infiltration of parasitized cells less than in jejunum. Where lymph follicles occur, parasitized clasmatocytes are numerous in the neighboring submucosa.

Lungs. In the pneumonic areas the alveoli contain blood and leucocytes, among which are a few parasitized clasmatocytes. There is no endothelial proliferation.

Heart. In the myocardium are several areas in which the muscle cells are fragmented, and in these areas are collections of cells including polynuclear leucocytes, lymphocytes, plasma cells, and clasmatocytes. The latter are heavily laden with Leishman-donovan bodies. No endothelial proliferation or other abnormalities.

Kidneys. A few parasitized cells seen in lumina of capillaries in glomeruli and interstitial tissue. A number of convoluted tubules contain blood. Most of them are somewhat distended but only a few contain casts.

Testis. In the loose interstitial tissue are many single clasmatocytes more or less heavily parasitized (Fig. 31). The so-called "cells of Leydig" may be distinguished from clasmatocytes by the presence of more chromatin in the Leydig cell nuclei, the deeper staining of their cytoplasm, which is often of a bronze color, and the sharper cell outline. Since both our monkeys were females, we have had no opportunity to corroborate directly the statement of Shortt (6) that, in the monkey, the Leydig cells become parasitized with *Leishmania.* In the seminiferous tubules spermatogenesis is much decreased, only a few cells beyond the spermatogonia stage being present. Sections of prostate, urinary bladder, pancreas, and aorta are normal.

Discussion

The development of the tissue reaction in the hamsters is directly proportional to the duration of infection and to the multiplication of the parasites. Endothelial proliferation and the development of "clasmatocyte tissue" in the reticular connective tissue of favorable organs are the two main features of this reaction. The liver is the main site of the purely endothelial reaction; the bone marrow is the

best illustration of the interstitial reaction. Where both elements in the reaction may operate, as in the spleen, lymph nodes and stroma of the intestinal mucosa, the accumulation of parasitized cells is greatest. The remarkable tolerance of the hamster to an intense infection permits the accumulation of parasitized cells in practically every organ of the body where a reticular connective tissue is present. The only parenchymatous cells which become invaded, according to our observations, are those of the liver and the adrenal cortex.

In the two monkeys studied, although death was apparently imminent as a direct result of *Leishmania* infection, the tissue reaction was much more prominent than the parasitic invasion. We have considered the possibility that the organisms failed to stain in the spleen and lymph nodes as well as they did in those organs in the hamsters, but have discarded this possibility because of the satisfactory staining secured in the liver, lungs, and bone marrow of the monkeys. The destruction of both clasmatocytes and Leishman-donovan bodies in the spleen and lymph nodes may be related to the functions of those organs, and the products of this destruction may have been partly responsible for the rapid course of the disease in these animals. The remarkable amount of endothelial proliferation in the liver and lungs of the monkeys, practically occluding many capillaries in both organs as well as some of the veins in the lungs, may also have been an important factor in the progress of the disease. Although endothelial proliferation in the lungs was not very marked in the hamsters and was absent in the human cases, its intensity in the monkeys is in keeping with the well-known function of these cells to act as scavengers for carbon, blood pigment, and other foreign substances found in the parenchyma of those organs.

The two human cases here reported, although not completely studied, showed essentially the same lesions as the experimental animals. Specific treatment and terminal infections may have caused some alterations in the picture. It is well known that acute infections like pneumonia will cause remission of the symptoms of kala-azar, with reduction in the size of the spleen and inability to recover *Leishmania* from spleen puncture. Nevertheless, in the two cases reported "clasmatocyte tissue" was well developed in the spleen, in some lymph nodes, and in the bone marrow, and the endothelial reaction in the liver was typical. If tissues from the intestine and mesenteric lymph nodes in Case 2 could have been carefully secured

immediately after death, the picture produced in the hamsters would probably have been more accurately duplicated.

The histological picture produced in the cutaneous form of leishmaniasis is essentially the same as that in the visceral form of the disease as here described. Wright [2] stated in his original description of "tropical ulcer" that "these large cells, over extensive areas, are very numerous and constitute the principal part of the infiltration." The local skin lesion might therefore be termed a "clasmatocytoma," which becomes an ulcer only when the skin surface is broken, and a granuloma only when the ulcer is in the process of healing.

Lesions involving mainly the capillary endothelial cells and the clasmatocytes are not limited to kala-azar, but in no other disease is the intensity of the reaction nearly so great.* Typhoid fever probably comes nearest to kala-azar in this type of reaction. In fact the histological picture of that disease as described by Mallory [10] could almost be duplicated by the cellular reaction in the intestine, mesenteric lymph nodes, spleen, and bone marrow of a moderately severe case of kala-azar. The liver lesions in the two diseases also have much in common. There are two main differences in the lesions of the two diseases. The first is the presence of the protozoan parasites in the cells in kala-azar. The second is the fact that the typhoid lesions, being stimulated by a relatively toxic organism, usually go on to necrosis, whereas those of kala-azar in which the invading organism is relatively non-toxic for individual cells, are mainly productive in nature, and never show necrosis of more than individual cells.

The descriptions by Councilman and his co-workers,[11, 12] of the lesions produced by diphtheria in the spleen and lymph nodes, and by variola in the spleen, lymph nodes, and bone marrow also involve chiefly the clasmatocyte and are very similar to those seen in kala-azar.

* Since completing this paper, my attention has been called by Dr. S. T. Darling to his work on histoplasmosis.[13] The lesions caused by the parasite *Histoplasma capsulatum* are almost identical with those produced by *Leishmania*. The parasitization and proliferation of endothelial cells are similar to the lesions in kala-azar, and the tubercle-like nodules of histoplasmosis, occurring in the lungs and intestine, correspond closely to the localized lesions of cutaneous leishmaniasis. Since no more cases of the disease have been encountered since Darling's original report, it remains somewhat of a mystery. Darling at first considered the parasite *Histoplasma capsulatum* to be a protozoön, but he now agrees (personal communication) with da Rocha Lima [14] that it is probably a Cryptococcus, closely related to *Cryptococcus farciminosus*, which causes epizoötic lymphangitis in horses.

In the study of cells by observing their ability to take up vital dyes and harmless particles, such as carbon, the clasmatocyte has received a great deal of attention. Most of the work done with these substances consists of single or repeated injections of the material to be used as an indicator of cell activity, followed by histological study of the cells in sections or smears. Foot,[15] for instance, made histological studies of these cells in tissue sections after a series of intraperitoneal injections of trypan blue, intravenous injections of lampblack and subcutaneous injections of agar. Simpson [16] studied these cells in the circulating blood after repeated intravenous injections of various colloidal dyes and particulate suspensions. Kala-azar offers another method of studying these cells, and the present study has revealed the development of the cells involved into a fixed tissue which is probably one expression of the function of such cells. We are dealing with a parasite of very low virulence which selects the clasmatocyte, or is selected by it, and which multiplies within the cell but destroys it only by its numbers, not apparently by toxins. At the same time it stimulates the specific cell to local multiplication, thereby furnishing a factor not supplied by foreign bodies, such as carbon particles. It may be that further study of the disease in experimental animals will add more to our knowledge of the function of the clasmatocyte.

Summary

1. The hamster, *Cricetulus griseus*, is an ideal animal for the experimental study of kala-azar because of the ease with which it can be infected and because of its tolerance of an intense infection.

2. The specific tissue reaction to the infection consists of endothelial proliferation and the formation of solid masses of "clasmatocyte tissue." The liver, spleen, lymph nodes, and bone marrow are the chief sites of formation of this tissue. The formation of tissue masses often appears to be in advance of the parasitization of individual cells.

3. In the most advanced infections the parenchymatous cells of the liver and adrenal cortex become parasitized, and severe degeneration of the liver occurs. The spleen reaches huge proportions, lesions appear in the kidneys, and parasitized clasmatocytes appear in the connective tissue of practically all organs and tissues.

4. In advanced infections the stroma of the intestinal mucosa is

the site of massive accumulation of parasitized cells. Contrary to Perry's observations in human cases, the epithelium covering parasitized villi is intact, if post-mortem trauma and autolysis of the mucosa are avoided.

5. In two monkeys experimentally infected, endothelial proliferation dominated the pathological picture, leading to practical occlusion of capillaries and veins of the liver and lungs.

6. Two human cases show practically the same histological picture as that seen in experimental animals. Treatment by tartar emetic and the influence of terminal infections are probably responsible for the differences observed.

7. In a few other diseases, notably in typhoid fever, the clasmatocyte is the principal cell involved in the tissue reaction. In typhoid fever, however, the toxicity of the invading organism prevents the development of so productive a lesion as that seen in leishmaniasis.

8. The further study of tissues from animals experimentally infected with *Leishmania* may contribute to our knowledge of the origin, activities, and functions of the clasmatocyte.

ACKNOWLEDGMENT

The author wishes to express his appreciation to Doctors C. W. Young and H. J. Smyly for furnishing the tissues of hamsters used in this study; to Professor Ralph G. Mills for permission to report the two human cases; to Doctor Simon Flexner for providing accommodations at the Rockefeller Institute, New York, in which to finish the paper; and to Mr. Schmidt of the Department of Illustration, Rockefeller Institute, for taking several of the photomicrographs.

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DESCRIPTION OF PLATES XXIII–XXVIII

Fig. 1. The hamster, *Cricetulus griseus*. About 2/3 life size.
Fig. 2. Liver of 125-day hamster. Endothelial cell containing Leishman-donovan bodies and projecting into lumen of central lobular vein. x 605.
Fig. 3. Liver of 125-day hamster. Parasitized clasmatocytes free in lumen of central lobular vein. x 512.
Fig. 4. Liver of 125-day hamster. Portal space crowded with parasitized clasmatocytes. x 251.
Fig. 5. Liver of 351-day hamster. Capillary occluded by a mass of parasitized endothelial cells. Adjacent liver cells also parasitized. x 745.
Fig. 6. Liver of 351-day hamster. Parasitized liver cell and mitoses in adjacent endothelial cells. x 1248.
Fig. 7. Liver of 351-day hamster. A group of endothelial cells, one heavily parasitized, another in mitosis. x 1248.
Fig. 8. Liver of 351-day hamster. Heavily parasitized endothelial cells in a central lobular vein. x 745.
Fig. 9. Liver of 455-day hamster, showing disintegration of liver tissue and huge parasitized endothelial cells. x 84.
Fig. 10. Liver of 455-day hamster. Liver cells compressed between huge parasitized endothelial cells. x 745.
Fig. 11. Spleen of 125-day hamster. Heavily parasitized cells in pulp and blood sinuses. x 745.
Fig. 12. Spleen of 125-day hamster, showing "clasmatocyte tissue" partly replacing a lymph follicle. x 130.
Fig. 13. Spleen of 455-day hamster, showing "clasmatocyte tissue" occupying most of the spleen substance. x 130.
Fig. 14. Spleen of 455-day hamster. Huge parasitized clasmatocytes in spleen pulp. Megakaryocyte, without Leishmania, stands out in contrast. x 745.

Fig. 15. Spleen of 455-day hamster. Parasitized cells partly lining blood sinus. One cell in sinus contains both red blood cells and a Leishman-donovan body. x 745.

Fig. 16. Lymph node of 90-day hamster. "Clasmatocyte tissue" invading lymph follicles from periphery of node. x 84.

Fig. 17. Lymph node of 125-day hamster. A multinucleated clasmatocyte containing many Leishman-donovan bodies. x 1248.

Fig. 18. Bone marrow of 125-day hamster. "Clasmatocyte tissue" at center of a small tongue of marrow in head of femur. x 233.

Fig. 19. Bone marrow of 455-day hamster. "Clasmatocyte tissue" comprises about one half of the marrow. x 84.

Fig. 20. Adrenal of 455-day hamster. Gland cell of cortex containing Leishman-donovan bodies. x 1864.

Fig. 21. Parasitized clasmatocytes in stroma of fat outside epididymis of 351-day hamster. x 1025.

Fig. 22. Duodenum of 455-day hamster. Villus, with stroma filled with parasitized clasmatocytes. x 419.

Fig. 23. Prostate of 455-day hamster. Submucosa filled with parasitized clasmatocytes. x 419.

Fig. 24. Appendix of Monkey 1. Lymph vessel in serosa containing parasitized clasmatocytes. x 810.

Fig. 25. Lung of Monkey 1. Capillary in alveolar wall, occluded by parasitized clasmatocytes. x 932.

Fig. 26. Liver of Monkey 2. Intense endothelial proliferation occluding a blood capillary. A number of lymphocytes and plasma cells are present among the endothelial cells. No typical Leishman-donovan bodies are found. x 419.

Fig. 27. Lung of Monkey 2. Interlobular vein entirely occluded by endothelial proliferation. x 251.

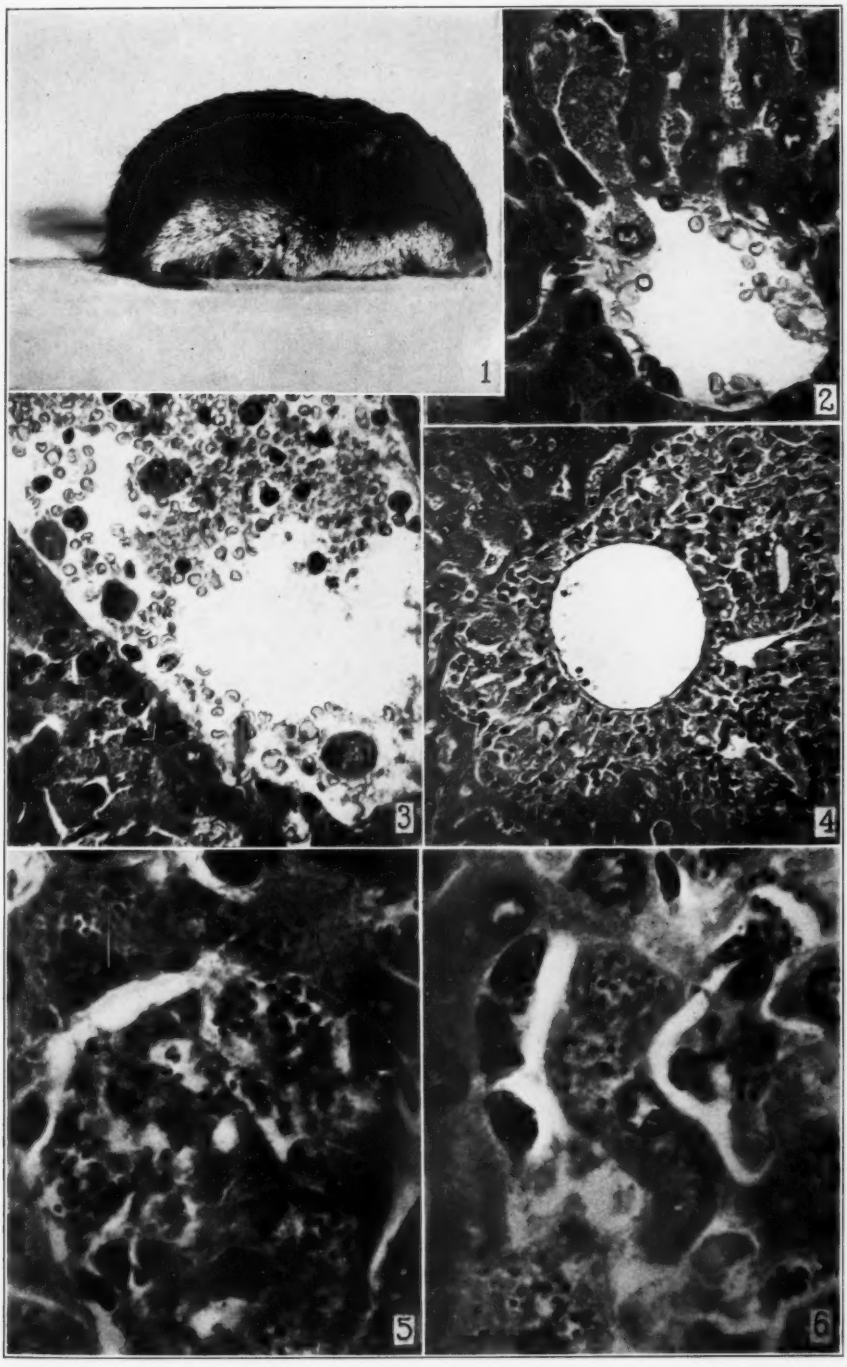
Fig. 28. Spleen of 138-day hamster. A mass of clasmatocyte tissue in which the cells toward the center contain very few Leishman-donovan bodies. x 783.

Fig. 29. Bone marrow of 110-day hamster. Some clasmatocytes contain no parasites. One contains both parasites and a red blood cell. x 1400.

Fig. 30. Lymph node of 351-day hamster. Clasmatocyte tissue partly replacing a lymph follicle. x 783.

Fig. 31. Human testis, Case 2. Parasitized clasmatocytes in the interstitial tissue. Note deep staining of nuclei and cytoplasm of cells of Leydig. x 783.

Figures 28 to 31 are colored camera lucida drawings. Tissue was stained with hematoxylin and eosin.



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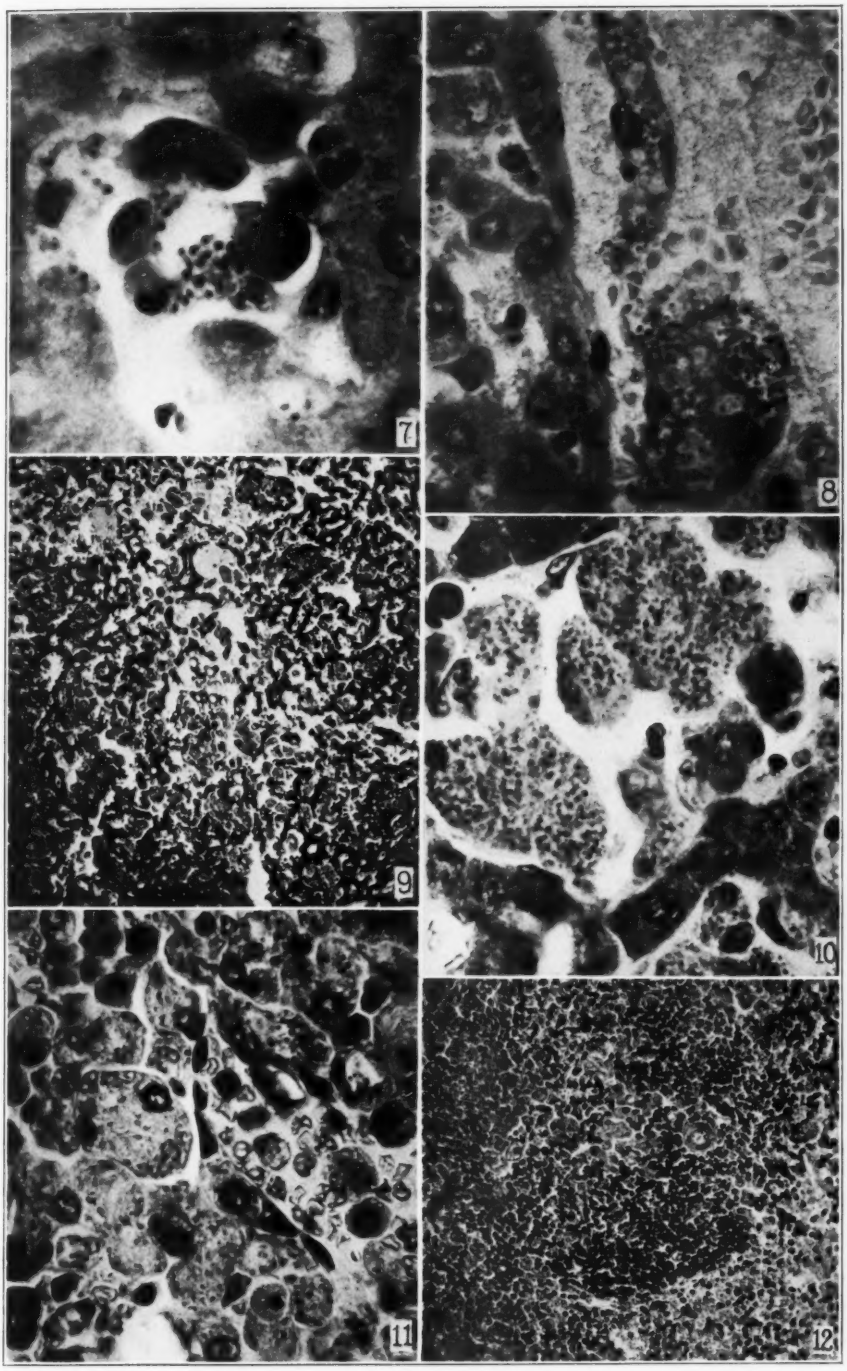

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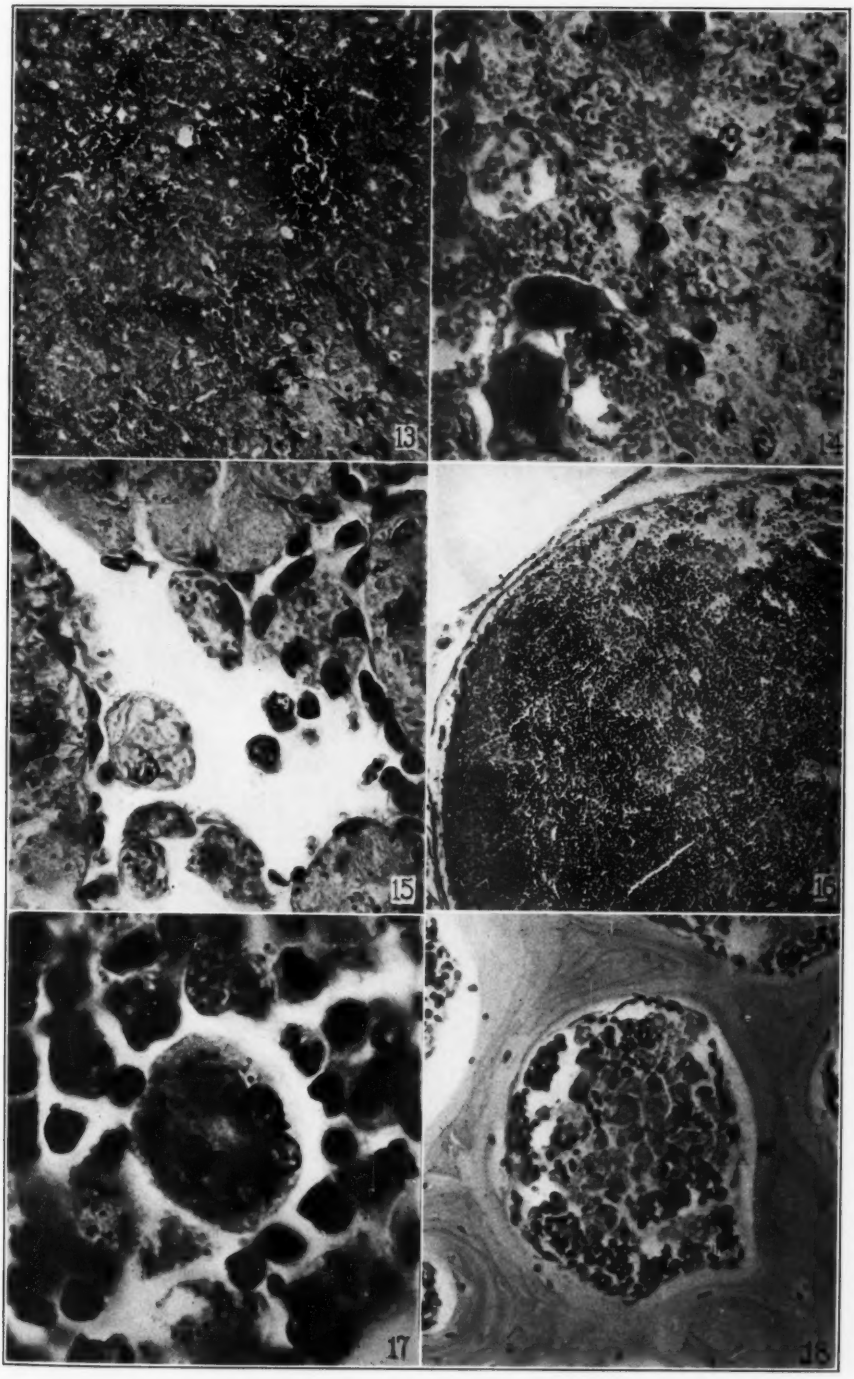

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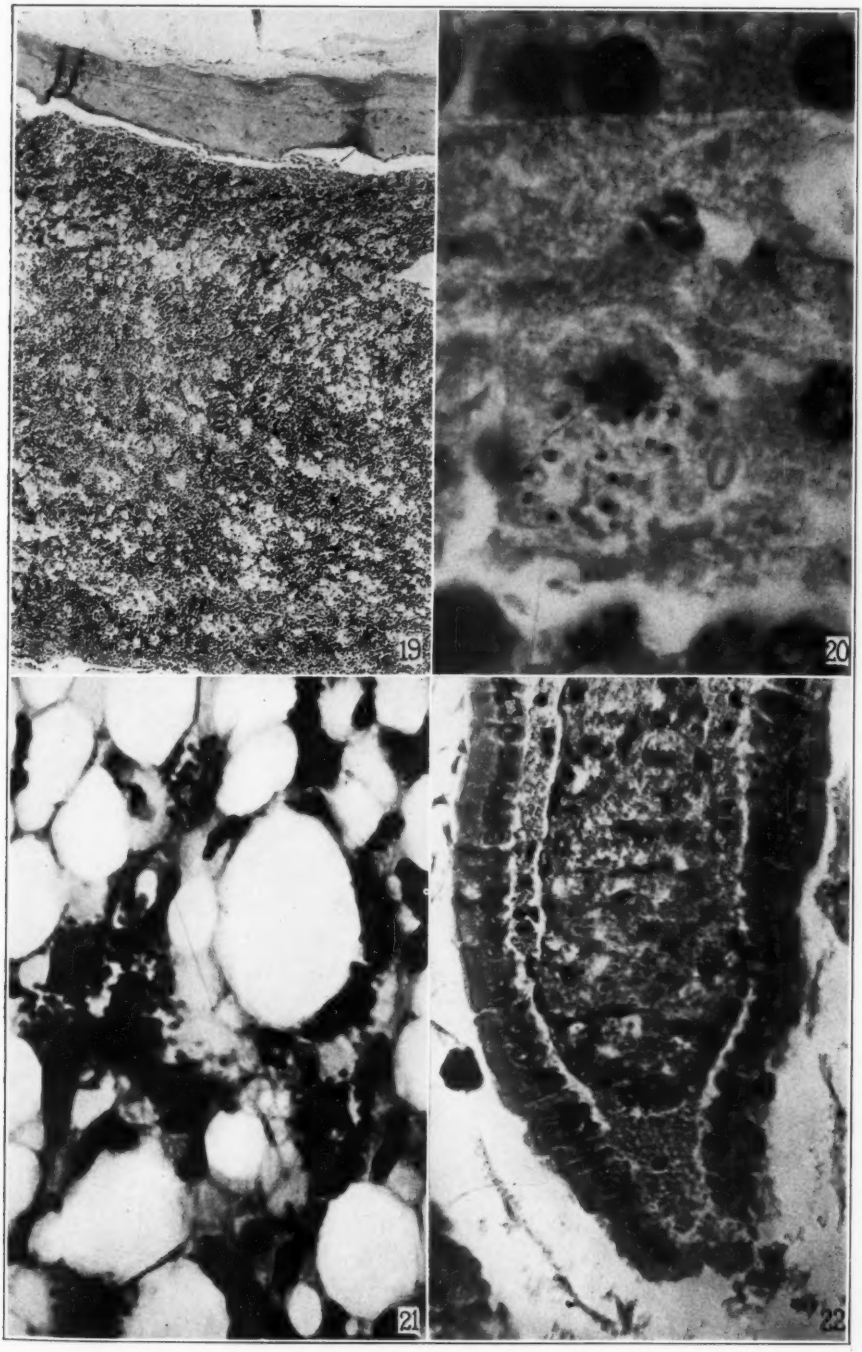

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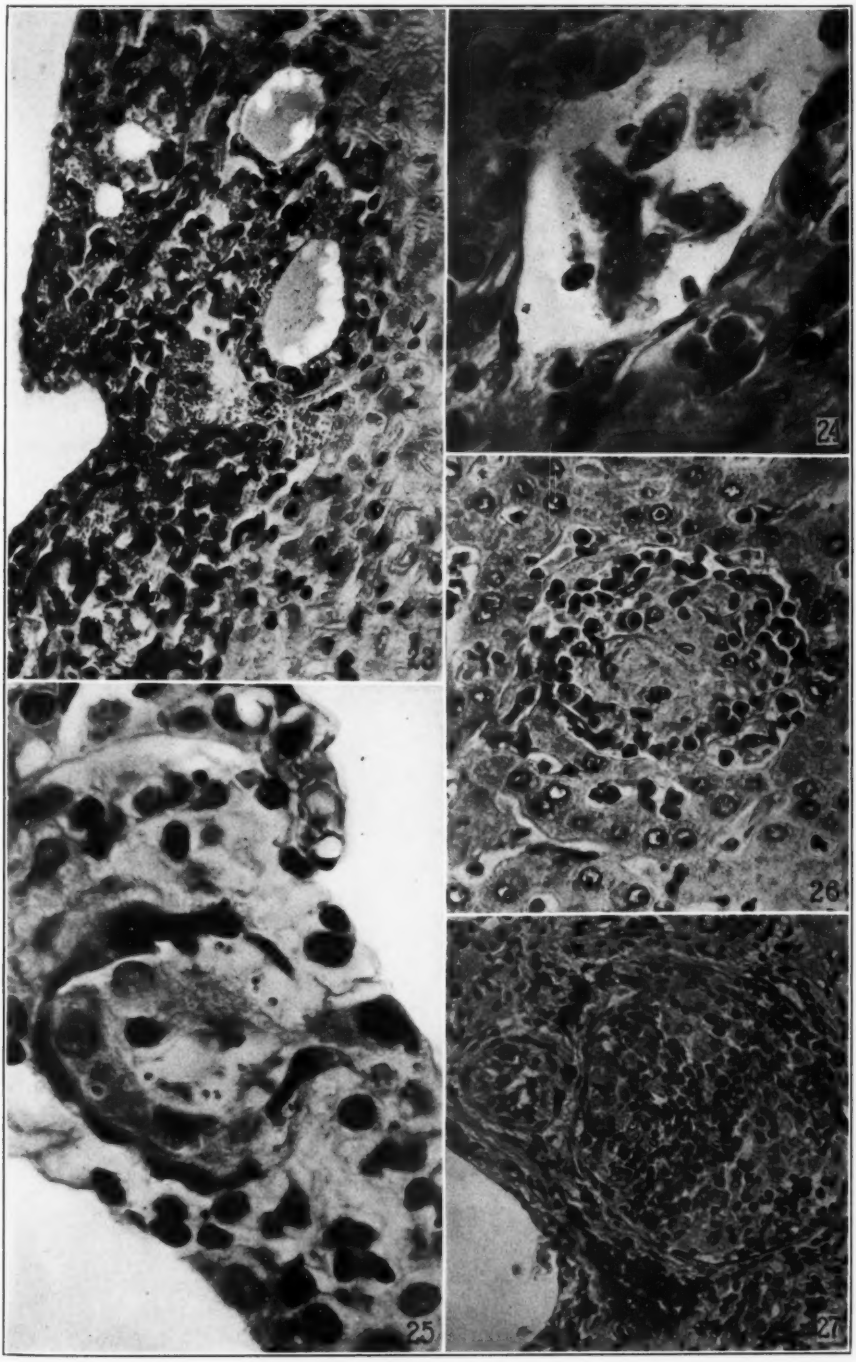

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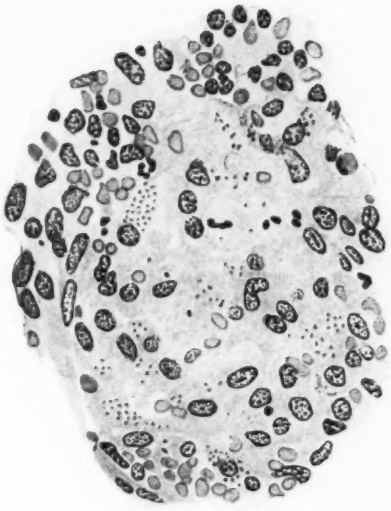

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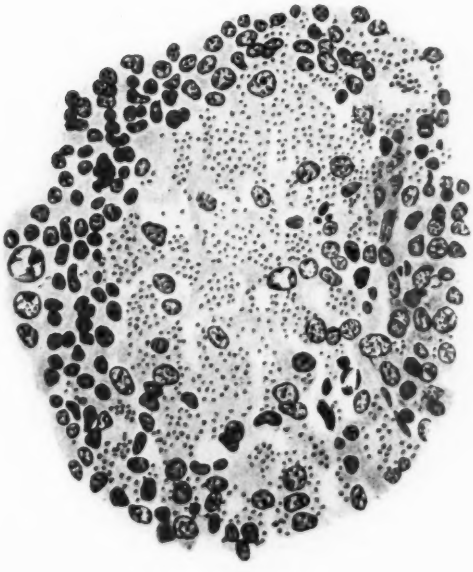

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PHAGOCYTOSIS OF ERYTHROCYTES IN THE BONE MARROW, WITH SPECIAL REFERENCE TO PERNICIOUS ANEMIA *

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The processes which bring about the destruction of red blood corpuscles have been studied intensively in man and in the lower animals by physiologists and pathologists, but our information regarding them still remains extraordinarily incomplete. The situation is presented clearly in the excellent review of the literature on the subject by Rous,[1] who discusses the various methods of blood destruction in different species and under normal and pathological conditions. Such a survey indicates the urgent need for more clinical observations and more animal experimentation directed towards the solution of the problem.

Among the processes which have been considered to account for the destruction of erythrocytes, that of phagocytosis by fixed tissue cells or by wandering cells has long been recognized, but, in spite of the fact that it has been frequently described as occurring under normal and pathological circumstances, it has not usually been regarded as a factor of particular significance in blood destruction in man. The recent publications of Aschoff [2] and Lepehne,[3] however, on the phagocytic cells of the so-called "reticulo-endothelial system" of the liver, spleen, lymph glands, hemolymph glands, and bone marrow, and their rôle in the pathogenesis of certain types of hemolytic jaundice, make it apparent that phagocytosis may, at least occasionally, become the predominant element in blood destruction. When one considers the relative bulk of the spleen and liver in comparison to that of the normal active bone marrow it is not surprising that the attention of investigators has been directed chiefly to these larger organs and that the bone marrow has received little consideration as a possible site of blood destruction. There are, however, pathological conditions in which the fatty marrow of the long bones becomes replaced by a cellular tissue and the bone marrow becomes an organ of material size. This happens, for instance, in

* Received for publication December 20, 1924.

pernicious anemia, and the fact that the hyperplastic bone marrow may be the seat of a striking amount of phagocytosis of red blood corpuscles has led us to a consideration of the part played by the bone marrow in blood destruction in this disease.

The occurrence of phagocytosis of red corpuscles in the bone marrow in pernicious anemia has been recognized ever since the earliest observations on the histology of the disease. Cohnheim [4] mentioned it in 1876, and in 1877 Osler and Gardner [5] described a case in which three or four cells, each containing five or six red corpuscles, were found in a single field of the vertebral bone marrow. Riess,[6] writing in 1881, was greatly impressed with the large numbers of phagocytic cells in the bone marrow in pernicious anemia, and raised the question of their relation to the pathogenesis of the disease. Dickson[7] says, "Pigment cells, and cells containing erythroblasts and red corpuscles are seen in very great numbers, for example, in pernicious anemia . . ." It is unnecessary to cite the extensive literature in which similar observations have been made, for the existence of phagocytosis in the bone marrow in pernicious anemia is commonly accepted. MacCallum [8] mentions it and illustrates it in his "Text Book of Pathology." With few exceptions, however, the writers have contented themselves with incidental references to the process and little attention has been paid to its possible relation to the pathogenesis of the disease. This aspect of the subject assumes some importance in the light of the current hypothesis, based largely on the abnormal increase of bilirubin in the blood serum and in the bile, that pernicious anemia is associated with an increased blood destruction. The failure to account for this excessive blood destruction satisfactorily in any other way raises the question as to whether the phagocytosis of erythrocytes, so frequently observed in the bone marrow, may be a factor in producing the destruction. The answer to this question depends in part, at least, on whether phagocytosis is more prominent in the bone marrow in pernicious anemia than in other diseases and on the constancy of its occurrence in pernicious anemia.

The phagocytic cells of the bone marrow arise either from the vascular endothelium or from the reticulum.[7] With the material and stains available it has not been considered possible to differentiate accurately between these two types of cell and no attempt has been made to do so. In all probability the majority of the phago-

cytes, and certainly those which are free, wandering cells, are to be classed as "endothelial leucocytes" in the nomenclature of Mallory [9] or as "clasmatocytes" in the nomenclature of Sabin.[10]

In this investigation a distinction has been made between the presence in a phagocyte of erythrocytes containing hemoglobin and the presence of granules of so-called "hemosiderin" in which the iron is in such a form as to give the Prussian blue reaction. This distinction seems to be justified because certain diseases, such as pernicious anemia, are characterized by a predominant phagocytosis of hemoglobin-containing cells, while others, such as hemochromatosis, are marked by the presence of large amounts of hemosiderin within the cells, and still others, such as cirrhosis of the liver, frequently show both features to a more or less similar degree. In many instances, special stains for iron have been made but the yellow-brown or yellow-green color of the latter pigment is so characteristic that they are not usually necessary. There can be no question that the hemosiderin is derived from hemoglobin but it is more difficult to determine whether this pigment has been formed within the phagocyte or whether it was already formed when taken up by the phagocyte. As far as it goes, the histological evidence indicates that in most cases certainly the bone marrow phagocyte ingests the red corpuscles and that the hemosiderin is formed from hemoglobin within the phagocyte. This is suggested by the presence of hemoglobin-containing cells and granules of hemosiderin in the same phagocyte and by the fact that phagocytes which contain erythrocytes of normal appearance may also contain masses, staining for iron, of the size and shape of a red corpuscle. These masses apparently represent the early phases in the transformation of hemoglobin into hemosiderin — perhaps before the complete dissolution of the stroma of the red corpuscle.

The relative extent to which hemosiderin granules or hemoglobin-containing red corpuscles are found in the phagocytes may merely indicate the capacity of the phagocytes for retaining or storing the ingested material, but it is also quite possible that it indicates the intensity or severity of the phagocytic process. The fact that the phagocytosis of hemoglobin-containing red cells is particularly prominent in certain diseases associated with anemia and jaundice would seem to make such an hypothesis quite plausible. If the pigment remains in the phagocyte for a certain length of time it is

probable that the change from hemoglobin to hemosiderin occurs. Rich [11] states that the splitting of hemoglobin into bile pigment and an iron-containing residue by phagocytic cells of mesodermal origin, in tissue cultures, can be watched under the microscope. Pearce,[12] reviewing his own work and that of Karsner, Amiral, and Bock,[13] on the production of phagocytosis of erythrocytes in the lymph nodes of splenectomized animals by the injection of hemolytic sera, says that the phagocytosis "reaches its height somewhere between twelve and twenty-four hours after injection, and then the destruction of corpuscles goes on, so that at forty-eight hours there is nothing left but pigment and corpuscular fragments. Furthermore, with the passage of time the individual phagocytes become more and more filled with erythrocytes until about twelve to twenty-four hours, at which time there is a disappearance of the erythrocytes with the substitution of the pigment granule." If this evidence can be accepted as throwing a general light on the situation in man, one is justified in assuming that phagocyted red corpuscles which contain unchanged hemoglobin have probably not been inside the phagocyte for more than twenty-four hours. Thus the association of a histological lesion showing extensive phagocytosis of hemoglobin-containing erythrocytes, together with an increase of the amount of bilirubin in the blood plasma would indicate an acute destructive process in which large numbers of erythrocytes have been ingested by phagocytes and have been again transferred, probably partially destroyed, into the blood stream before the change from hemoglobin to hemosiderin has had time to take place. On the other hand, the presence of a large amount of hemosiderin, with or without accompanying erythrocytes, in the phagocytic cells would suggest a much less acute phagocytic process, and the relative proportion of hemoglobin-containing erythrocytes to hemosiderin granules would give an indication of the rate of blood destruction and of pigment change.

Control Observations

In order to determine the frequency and extent to which phagocytosis of red blood corpuscles occurs in the bone marrow in conditions other than pernicious anemia, observations have been made on four specimens of normal vertebral bone marrow and on sections of bone marrow (mostly vertebral) from 130 autopsies of cases dying

in a general hospital. The latter were, with very few exceptions, unselected and represent the various types of disease which are usually seen in the Pathological Service of such an institution. The amount of material and the wide range of diagnosis is thus sufficient to give at least a general conception of the frequency and extent to which phagocytosis occurs in the bone marrow in man. Grohé,[14] in 1884, and Geelmuyden,[15] in 1886, reported similar studies on the bone marrow. Both of them observed phagocytosis in a variety of conditions, and neither felt that it was much more extensive in the few cases of pernicious anemia examined than in a number of other diseases.

All of the sections of the vertebral bone marrow from four normal adults, in whom death was the result of traumatism, showed the presence of intracellular hemosiderin granules. In two instances only occasional pigment granules were found; in the third there was a considerable number of phagocytes containing coarse pigment granules — an amount that under other circumstances might have been considered to be abnormally great. In only one specimen were any phagocyted erythrocytes seen, and in this instance they were extremely rare. They were apparently intact and contained unaltered hemoglobin. From these few observations one can merely conclude that phagocytosis of erythrocytes occurs normally in the human bone marrow, but that it occurs only to a very limited extent. In this connection it is worth noting that Van den Bergh [16] has found bilirubin to be present in the sera of many normal individuals and the suggestion may be made that the normal variations in bilirubin depend on the activity of the phagocytic cells of the bone marrow and of the other organs of the reticulo-endothelial system.

In the study of the specimens of bone marrow from 130 patients dying in the hospital from various causes, an attempt has been made to estimate the extent to which erythrocytes and hemosiderin granules are present in the phagocytic cells. It is, of course, appreciated that this cannot be done with any high degree of accuracy because quantitative methods are not applicable, but it has been satisfactory to find that observations on the same specimen at widely separated intervals of time have been essentially in agreement. Specimens from different parts of the bone marrow in the same case may vary somewhat in histological picture but the differ-

ence is not sufficient to militate against the general value of observations on one or two sections. It is felt that the observations reported may be taken as rough representations of the amount of phagocytosis present. No further accuracy is claimed for them, and no further accuracy is necessary for the purpose in view.

In almost every case phagocytic cells containing either erythrocytes or pigment, or both, were found, and the impression was received that sufficiently extensive study would have revealed them in all. In seventy-seven out of the 130 cases the number of phagocytes containing either cells or pigment was not considered to be outside of what may be generally accepted as the normal limits. In twenty-one cases the phagocytosis was regarded as probably abnormal, and in thirty-two other cases the amount of phagocytosis undoubtedly exceeded the normal. In fourteen of the latter group pigment granules were present in the phagocytes to a relatively greater extent than were hemoglobin-containing erythrocytes. However, ingested red corpuscles were present in all specimens and usually in numbers greater than normal. The diagnoses in this group were — cirrhosis of the liver in three cases, hemachromatosis, adhesive pericarditis and perihepatitis, osteitis deformans and lobar pneumonia, lobar pneumonia and toxic jaundice, tuberculosis (two cases), carcinoma of esophagus, aplastic anemia and subdural hemorrhage, chronic myocarditis and chronic nephritis, actinomycosis, and Paget's disease. In twelve of the thirty-two cases there was a definitely excessive phagocytosis of erythrocytes, accompanied, in most instances, by a small or moderate number of hemosiderin granules. The diagnoses in this group were — bronchopneumonia or lobar pneumonia in six cases (with lung abscess and empyema in one case), typhoid fever, tuberculosis in two cases, mitral stenosis with thrombosis of left auricular appendage and multiple infarcts, and aplastic anemia and endometritis. One other case (24–112), which was studied incompletely in the clinic, as the patient died of a cerebral hemorrhage shortly after admission, is included here although the clinical record and the autopsy make it probable that he also had pernicious anemia. In the remaining six of the group of thirty-two cases both erythrocytes and pigment granules were present in the phagocytes to an abnormal extent. The diagnoses in these cases were — cirrhosis of the liver, chronic nephritis and healed perihepatitis, lobar pneumonia, bronchopneumonia and en-

cephalitis (?), gastric ulcer with perforation, and embolus of basilary artery. It is thus apparent that in a great variety of pathological conditions one may find a considerable number of erythrocytes and an abnormal amount of hemosiderin in the phagocytic cells of the bone marrow. It is impossible to classify these conditions, but the number of cases with liver lesions is rather striking. All five specimens of bone marrow from cases of cirrhosis of the liver showed red cells, pigment, or both, within the phagocytes. Beyond this one can only call attention to the incidence of increased phagocytosis in infections such as pneumonia, typhoid fever, and tuberculosis, in which the phagocytosis of cells is usually predominant. Similar findings have been described by Longcope [17] in typhoid fever and by Dickson [7] in pneumonia. While an excessive amount of phagocytosis was not observed in all of the cases of pneumonia or typhoid fever studied, it is of interest to call attention to the fact that certain cases of pneumonia and typhoid fever are associated clinically with slight degrees of jaundice, and Broun has found in some of these cases a "delayed" direct diazo reaction (Van den Bergh) which indicates that the jaundice is of hemolytic rather than of hepatic origin. It is quite probable that the hemolytic jaundice is the result of the phagocytosis. In cirrhosis of the liver Broun found the "prompt" direct diazo reaction, which is associated with jaundice of hepatic origin, but if the two types of jaundice, hepatic and hemolytic, were simultaneously present, the former would always mask the latter so that it would not be recognizable by this test.

An abnormal amount of phagocytosis is frequently observed in specimens of bone marrow which are hyperplastic, in the sense that there is an increase of cells at the expense of fat, but there is no definite relation between the degree of hyperplasia and the extent of the phagocytosis, and phagocytosis may be increased in specimens of bone marrow that are definitely not hyperplastic.

Pernicious Anemia

Eleven cases of pernicious anemia which have received careful clinical study form the basis of this report. Eight of them were patients at the Boston City Hospital and three at the Peter Bent Brigham Hospital. For the clinical records of the latter we are indebted to Dr. H. A. Christian, and for the pathological records and material to Dr. S. B. Wolbach. Ten of these cases may be said

to have died in an acute stage of the disease, although one of them (Case 2) also had a terminal pneumonia. The red corpuscle counts taken shortly before death varied between 500,000 and 1,000,000 per c.mm., and the hemoglobin variations were between 10 and 24 per cent in nine of the patients. In one case (Case 7) the hemoglobin was 55 per cent and the erythrocyte count was 1,200,000

TABLE 1

Cases of Pernicious Anemia

Case No.	Age	Per Cent Hemoglobin	Red Cells Million	Transfusions		Phagocytosis in Bone Marrow		Remarks
				Total Number	Last before Death	Hemosiderin	Erythrocytes	
1	64	19	1.0	2	14 days	+	+++++	
2	64	10	0.5	2	7 months	+	++++	
3	45	17	0.8	9	4 days	+	++++	Lobar pneumonia (terminal)
4	39	20	1.0	4	17 days	+	+++	
5	58	16	0.5	2	1 month	+	++++	
6	63	24	0.8	4	1 day	+	+++±	
7	55	55	1.2	0	.....	+	+++±	Last hemoglobin determination was 13 days before death
8	65	10	0.8	1	5 weeks	+	+++±	
9	52	16	1.0	0	.....	+	+++	
10	67	14	0.6	4	3 months	+±	+++±	
11	37	50	2.0	0	.....	±	+	Lobar pneumonia

thirteen days before death. On the day before death the erythrocyte count was 1,080,000 per c.mm. The bilirubin in the blood plasma was between 1.3 and 4.0 mgm. per 100 c.c. of blood in four of the cases just before death, and was 0.8 and 2.0 mgm. in Cases 1 and 6 just after death. The eleventh case is considered separately from those dying in an acute stage of the disease because death was due to lobar pneumonia contracted during a remission of the pernicious anemia, at a time when the red cell count was 2,000,000 per c.mm. and the hemoglobin was 50 per cent. As will be seen, the histological findings in the bone marrow in this case were quite different from those in the cases dying in an acute stage of pernicious anemia.

Two important features characterize the phagocytic process in

the bone marrow in the first ten cases. These are the extraordinarily great amount of phagocytosis of erythrocytes and the relatively slight amount of hemosiderin present in the cells. The iron-staining pigment is not at all prominent and often scarcely exceeds the quantity that may be present in normal bone marrow. A part of it is deposited in very fine granules in the endothelium lining the vascular sinuses and a few small masses may be extracellular. The rest is in phagocytic cells, and is usually interspersed between the ingested red corpuscles. The extent to which erythrocytes have been phagocyted, on the other hand, is, as far as can be roughly estimated in eight cases, greater than in any of the control cases, and in two cases about equal in degree to the half dozen control cases which showed the largest amount of phagocytosis of cells. With one exception, however, (24–112, probable pernicious anemia) all of these control cases showed much more pigment than did the cases of pernicious anemia.

The phagocytosis of erythrocytes usually takes place in free, round or oval cells (endothelial leucocytes; clasmatocytes) with large vesicular nuclei which become compressed, often curved, and displaced toward the periphery of the cell when many red corpuscles have been ingested. If a single erythrocyte has been ingested it often lies within the concavity of the nucleus. Phagocytosis may also occur in cells with slightly eosinophilic cytoplasm and delicate cytoplasmic processes. The number of phagocytic cells varies in different parts of the same section but there are often as many as three to six in a single high-power field. The number of erythrocytes ingested by a single phagocyte is also very variable and runs from one to three up to twenty or more. When one takes into consideration the fact that in pernicious anemia the cellular, hyperplastic bone marrow is a wide-spread, extensive tissue which fills the long bones as well as the short bones and which even brings about an enlargement of the marrow cavities within the long bones, it is apparent that the number of erythrocytes which are being phagocyted is enormous. The extent of the phagocytosis is indicated by Figures 1 to 4, each of which is from a drawing of the cells in a single oil-immersion field.

The ingested erythrocytes are for the most part strikingly normal in their appearance, both as regards size and shape. Small, abnormal, irregular forms and poikilocytes are often seen but they are,

on the whole, less common than normal red corpuscles. The hemoglobin usually stains exactly as does the hemoglobin in the red cells in the vascular spaces. Erythrocytes in which the pigment has been altered so that it has the appearance of hemosiderin or gives an iron stain are distinctly rare. Nucleated erythrocytes are very frequently found within phagocytes and occasionally leucocytes have been ingested.

The phagocytic cells described above are seen best in well-stained thin sections of bone marrow. They are, however, often somewhat difficult to distinguish in the densely cellular tissue and may be more easily recognized along the edge of the sections where the cells have been mechanically separated. In spite of being present in large numbers, they may well be overlooked in a routine study of the bone marrow unless attention is directed to this particular feature. The phagocytic cells are also readily found in fresh smears or in smears made from fresh emulsions of marrow in salt solution. They are quickly destroyed in smears dried in the air before fixation, but excellent preparations may be obtained by fixing the smears while still wet in Zenker's solution and then staining. Dickson[7] recommends this method of examination. Very satisfactory preparations may also be made by fixing in Zenker's solution the sediment of an emulsion of bone marrow in salt solution. These "wet fixed" smears may be stained with eosin and methylene blue or with one of the Romanowsky stains. The phagocytic cells are also easily seen in fresh, unstained emulsions of bone marrow in normal salt solution, and Dr. C. A. Doan has recently made a beautiful preparation of living bone marrow cells stained with neutral red and Janus green in which the phagocytic cells were particularly prominent.

Case 11, who, as mentioned above, died of pneumonia during a remission in the pernicious anemia, and in whom the relative inactivity of the process causing the anemia is indicated by a red corpuscle count of 2,000,000 per c.mm., and a blood bilirubin content of only 0.8 milligrams per 100 c.c., showed a histological picture in the bone marrow which was quite different from that described in the ten cases just referred to. There was very little phagocytosis either of erythrocytes or of pigment. The degree of phagocytosis in a single section could hardly be considered beyond the normal limits, although, of course, the process was going on throughout a tissue that is somewhat more extensive than the normal bone mar-

row. In this connection another point in the histology may be mentioned. Eight of the ten cases dying in an acute stage of pernicious anemia were almost without fat in the bone marrow, while two others (Cases 4 and 10) had only a slight amount of fat in the marrow. Case 11, on the other hand, had a very considerable amount of fat in the femoral bone marrow. This is unquestionably another indication that the pernicious anemia was undergoing a remission, for Zadek [18] has shown by examination of the marrow during life that during remissions in the disease the bone marrow tends to become more yellowish and less red in color.

Discussion

The observations on the bone marrow which have just been described indicate that phagocytosis of erythrocytes occurs to a very limited extent in normal marrow; that it may be considerably increased in a variety of pathological conditions; that in the active stages of pernicious anemia it occurs to a degree which, with rare exceptions, is not met with in other diseases, and that during a remission in the course of pernicious anemia it may cease to be a prominent factor in the bone marrow.

The first point for discussion with regard to the phagocytosis of erythrocytes in pernicious anemia is as to the character of the ingested cells. Are they foreign cells which have recently been introduced by transfusion? That they are not transfused cells is evident for a number of reasons: extensive phagocytosis of erythrocytes was frequently described long before transfusion became a common procedure; many of the ingested erythrocytes are young cells that still retain their nuclei; Case 2 of this series, with an enormous amount of phagocytosis, had not been transfused for nearly seven months; and Cases 7 and 9 had never been transfused. It is also of interest in this connection that in one of Dr. Channing Frothingham's patients who had received thirty-two transfusions, the bone marrow showed no phagocytosis of erythrocytes, but contained a very large amount of intracellular hemosiderin. The second question with regard to the character of the ingested erythrocytes is less easy to answer. Are they abnormal or inadequate cells? Is the phagocytosis merely a mechanism for ridding the circulation of worthless cells? The fact that the ingested cells look like entirely normal erythrocytes is,

of course, no evidence as to their functional efficiency. The occurrence, however, of so many nucleated red corpuscles among the phagocyted cells indicates that if these cells are indeed inadequate, they must become so very early. This is opposed to the observation of Wearn, Ames, and Warren [19] that the cells of a patient with pernicious anemia, transfused into another subject, continue to remain in the circulation as long as transfused normal cells. Such evidence certainly does not suggest that the erythrocytes in pernicious anemia are, as a whole, in any degree inadequate, but whether or not some of the red cells are rendered more liable than normal to phagocytosis in the patient with pernicious anemia is not known. The phagocytosis of erythrocytes in pernicious anemia must depend either on an alteration in the red cells which makes them more readily phagocyted or on a stimulus to the phagocytic cells which increases their avidity for erythrocytes. No convincing proof of either process can, however, be brought forward at the present time.

From the point of view of pernicious anemia the problem of immediate interest is whether this phagocytic process is an incidental matter, as it has generally been regarded, or whether it is a significant phase of the pathology of the disease. If the phagocytic process does have an essential relation to the pathology of the disease, this relation is, of course, limited to the hematological manifestations of the disease, namely, the anemia and the hemolytic jaundice. No other explanation of the cause of the anemia has been presented which is in any degree convincing and there are, on the other hand, certain facts which indicate that the phagocytosis may play a part in its production. The most striking of these is that in certain cases of pernicious anemia there is an easily demonstrated phagocytosis of erythrocytes in the bone marrow which is rarely equaled in degree in other pathological conditions. There can be no doubt that the phagocyted cells are ultimately destroyed and the amount of phagocytosis throughout the greatly increased bone marrow must account for the destruction of a very large number of erythrocytes. The histological picture of the bone marrow, with extensive phagocytosis of hemoglobin-containing erythrocytes, and with very little retention of hemosiderin in the phagocytes suggests an active hemolytic process. The hemoglobin or its decomposition products immediately after their formation probably pass quickly from the phagocyte into the blood stream. This would account for the finding

of hemoglobin and hematin, as well as bilirubin, in the plasma in acute stages of pernicious anemia, as reported by Broun, Ames, Warren, and Peabody.[20] Unfortunately the group of cases thus far studied satisfactorily, both clinically and pathologically, is extremely small but it is significant that the cases dying during an acute phase of the disease showed the greatest amount of phagocytosis of erythrocytes, while the case dying of pneumonia during a remission of the anemia showed by far the least amount of phagocytosis.

In this paper attention has been directed entirely to the phagocytosis of erythrocytes in the bone marrow. The bone marrow has been considered to be of particular interest because it is so large an organ in pernicious anemia, and also because phagocytosis is, at least as demonstrated histologically, a less considerable feature in the liver and spleen in this disease. The failure to cure pernicious anemia by splenectomy also indicates that the spleen plays, at most, a subordinate rôle in the production of the anemia. The bone marrow is not the only tissue, however, in which extensive destruction of red corpuscles takes place in pernicious anemia. In 1902 Warthin [21] published a paper on "The Pathology of Pernicious Anemia with Special Reference to Changes Occurring in the Hemolymph Nodes," and described extensive phagocytosis of erythrocytes in eight cases. One of his conclusions may well be quoted verbatim as it summarizes his point of view as to the hemolytic process in pernicious anemia. "The poison of pernicious anemia stimulates the phagocytes of the spleen, lymph and hemolymph glands, and bone marrow to increased hemolysis (cellular hemolysis). Either the phagocytes are directly stimulated to increased destruction of red cells, or the latter are so changed by the poison that they themselves stimulate the phagocytes. The hemolysis of pernicious anemia differs only in degree, not in kind, from normal hemolysis or the pathological increase occurring in sepsis, typhoid, etc."

Our own observations on the lymph nodes in pernicious anemia have, as yet, been limited in number but they are in entire agreement with those reported by Warthin. The studies on the bone marrow, described in this paper, taken in conjunction with the observations of Warthin, lend considerable support to his explanation of blood destruction in pernicious anemia by cellular hemolysis. Whether or not this method of blood destruction is the only factor in the production of the anemia, of course, remains an unsettled

question. It is quite possible that the hemolytic jaundice of pernicious anemia is the result of phagocytic action but that other factors take part in the causation of the anemia.

Conclusions

1. Phagocytosis of erythrocytes occurs in the normal bone marrow and to a much greater extent in a variety of pathological conditions. Among the latter the most notable are certain cases of cirrhosis of the liver, and of infectious diseases such as pneumonia, typhoid fever, and tuberculosis.

2. Phagocytosis of erythrocytes occurs in the bone marrow of patients dying in an acute stage of pernicious anemia in a degree rarely met with in other conditions. In a single case of pernicious anemia dying in a remission of the disease, phagocytosis of red blood corpuscles was not a striking phenomenon.

3. It is suggested that phagocytosis of erythrocytes may be a factor in the production of the hemolytic jaundice and of the anemia in pernicious anemia.

It would have been impossible for us to carry out this study without the very constant assistance of Dr. F. B. Mallory, Director of the Pathological Laboratory, and his group of associates. For their many kindnesses we are glad to express our deep appreciation.

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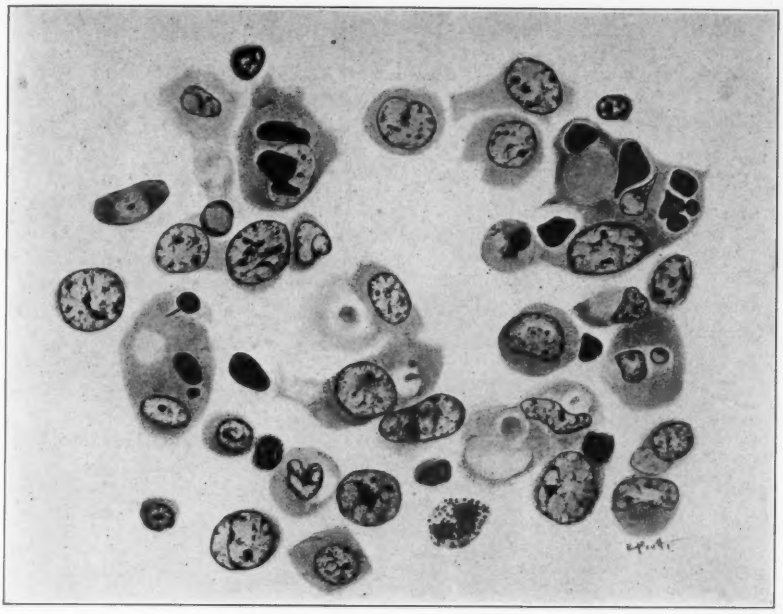
DESCRIPTION OF PLATES XXIX AND XXX

The illustrations are photographs, from drawings by Miss E. Piotti, of cells found in a single oil-immersion field.

Figures 1 and 2 are from smears of femoral bone marrow, and Figures 3 and 4 are from sections of femoral bone marrow.



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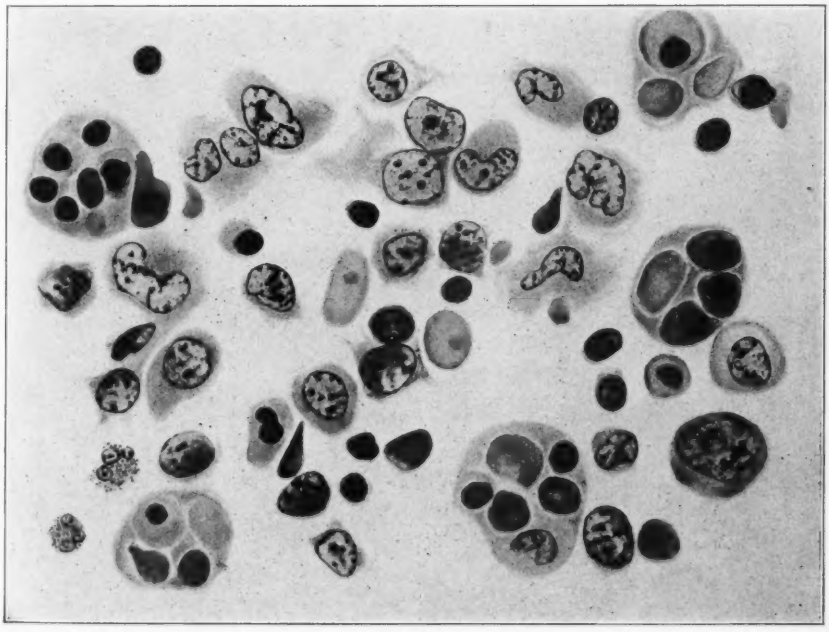

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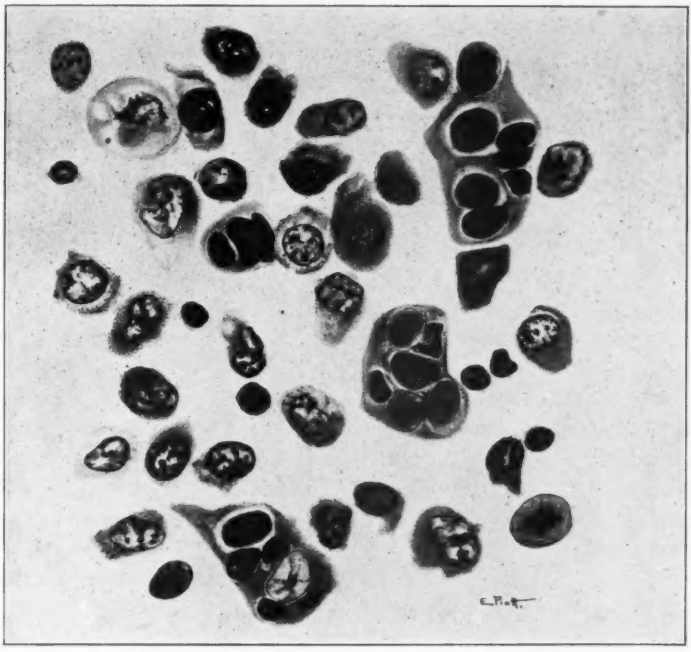

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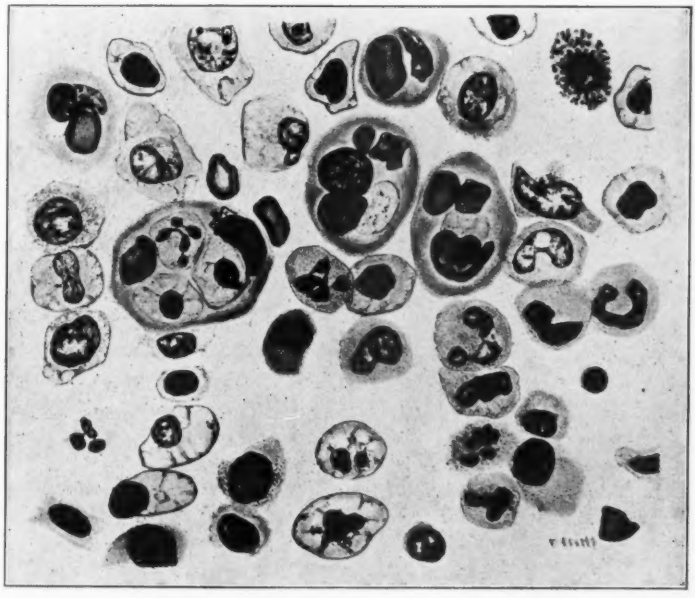

4

CONCERNING THE MICROSCOPIC STRUCTURE OF THE HYPOPHYSIS CEREBRI IN ACROMEGALY*

(BASED ON A STUDY OF TISSUES REMOVED AT OPERATION FROM 35 PATIENTS)

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If acromegaly is a disease entity, whether the lesion in the hypophysis be primary or secondary, the adenomatous change which takes place in this gland should show some constant histological characteristics capable of recognition. For the purpose of identifying these characteristics, if possible, and thereby explaining or interpreting the contradictions in the literature, we felt it would be fruitful carefully to re-examine the abundant material collected over a period of years in this clinic.

It is, of course, a fundamental principle that the microscopic investigation of the pathologic alterations of an organ demands a knowledge of its normal microscopic structure, which implies the use of certain special technical procedures by which the normal structure is best displayed. It is not sufficient to employ, as is still often done, simply stains to show nuclei and certain pathological substances such as fat. Many excellent methods for the study of the normal microscopic structure of the hypophysis have been developed, and although perhaps none of them is perfect in every respect, their neglect is doubtless the cause of much of the uncertainty and contradiction regarding the acromegalic gland.

NORMAL MICROSCOPIC DETAILS

It is a generally accepted view that the part of the hypophysis affected in acromegaly is the anterior lobe (*pars distalis*) and, in accordance with the principle just mentioned, a knowledge of its normal structural peculiarities is necessary. It may be said, at the risk of repeating common knowledge, that the pars distalis is composed of polygonal cells having, for the most part, sharply defined

* Read before the Boston Society of Neurology and Psychiatry, February 21, 1924. Received for publication December 26, 1924.

cellular boundaries. They are arranged in thick columns, between which wind delicate vascular sinuses.

The cells of the pars distalis are distinguished, by the type of granules which they contain, into acidophilic, basophilic and chromophobe cells. This terminology was based on the belief that one type of granules has an affinity for acid dyes and the other for basic dyes. It is now known that this belief was erroneous. It is preferable to speak of the granules as α and β granules, the α being easily understood to mean the acidophilic and the β to refer to the so-called basophilic (cyanophilic) granules. The cells which contain these granules may then be spoken of as α or β cells, for cells containing both types are not known to exist under normal conditions. The α and β cells are spoken of collectively as chromophile cells.

In addition, there are cells in the pars distalis which contain no granules. Some of them are chromophile cells which have lost their granules, but most of them have apparently failed to develop granules and are known variously as chromophobe, reserve, or chief cells. They often lie in the center of the cellular columns, although they may make up entire columns, especially in the periphery of the pars distalis or near the infundibulum. Often these reserve cells do not have distinct boundaries and their nuclei lying close together are known as nuclear clusters (Kernhaufen).

The α granules are large and distinct. They are stained intensely by eosin, acid fuchsin, neutral ethyl violet, etc. The β granules are finer and less distinct. They are also more difficult to stain, but alum hematoxylin, kresofuchsin, acid violet and aniline blue are capable of displaying them. There are also granules of a general nature, such as mitochondria and lipoid droplets, which may be demonstrated by special means.

The relative numbers of α and β cells in different hypophyses is most variable, even in different parts of the same pars distalis. The pattern which one finds may change completely in the space of a dozen serial sections. The relationship of the different cellular types to the blood vessels shows no constancy whatever.

Technical Procedures

All of our specimens were fixed immediately after removal from the body. The best fixative for the purpose is undoubtedly Regaud's fluid. Formalin-fixed material may be used if it is mordanted for

10 days in a 3 per cent aqueous solution of potassium bichromate before it is embedded. Alcohol, acids and sublimate mixtures are unsuitable. Acids (except osmic acid) and alcohol agglomerate the α granules, which are lipoidal, and prevent the demonstration of the finest ones, while sublimate produces artificial granulation of the cytoplasm which is apt to simulate β granules.

The α granules may be stained by many methods, among which may be mentioned: Benda's toluidin blue-sodium sulphalizerinate; methylviolet (M. B. Schmidt, Bizzozero-Launois); Heidenhain's or Regaud's iron hematoxylin; phosphotungstic acid-hematoxylin of Mallory; Kraus' hematoxylin; acid fuchsin of Altmann or Cagnetto; neutral gentian (Lewis); and neutral ethylviolet-orange G (Bailey). Eosin is totally unreliable for the identification of α granules. The best stain is neutral ethylviolet-orange G. It gives a sharply specific stain, a sharp contrast, and will not stain mitochondria. On material fixed in Regaud's fluid, Altmann's acid fuchsin and iron hematoxylin will always stain the mitochondria along with the α granules.

For the demonstration of the β granules on material fixed in Regaud's fluid only acid violet has been found to give a sharp stain (Bailey). On material fixed in formalin, kresofuchsin may be used. For specimens fixed in Zenker's fluid we have used alum hematoxylin.

For a sharp differential stain of the two types of granules in the same preparation we prefer the acid fuchsin-acid violet method of Bailey.[1] The neutral safranin-acid violet method of Lewis and Maurer [28] may be used on Regaud-fixed material provided the sections are passed for 3 minutes into 1 per cent aqueous solution of potassium permanganate and bleached in oxalic acid before being stained. We prefer the procedure of Lewis and Maurer to the earlier one of Bailey, in which the same combination of dyes is used.

The method described by Bailey for the differentiation of α granules and mitochondria in the same preparation has been found unreliable and abandoned. No other has been found to replace it. We have been obliged to depend upon a comparison of acid fuchsin and ethylviolet-stained preparations.

It has been necessary to use some material fixed in Zenker's fluid. For the identification of β granules in these tissues we have used alum hematoxylin. Since it errs on the side of too many

rather than too few granules, we feel certain that they are absent from our material. Many of the finer α granules are completely lost from Zenker-fixed material. We proved this conclusively and repeatedly where we had specimens fixed both in Zenker's fluid and in 10 per cent formalin from the same case. In the formalin-fixed material we could easily demonstrate α granules, while in the Zenker-fixed material they could be shown only incompletely with any of the methods at our disposal. We found, however, that the granules came out more sharply if the sections were mordanted in 3 per cent potassium bichromate before being stained.

Lack of a proper knowledge of staining methods explains many strange errors in the literature of acromegaly. Launois [29] gives in great detail a method of using methyl violet after Bizzozero, which he claims is a specific stain for β granules. Kon [25] falls into the same error with the method of M. B. Schmidt. Both methods stain α granules sharply and specifically as can be easily proved by trying them on an acromegalic adenoma known to contain only α granules. Huchard and Launois presumably used this method on their giant (see below, p. 200) thus unconsciously entering this case in the ranks of those having a pure eosinophilic adenoma, while in the literature it is often quoted, erroneously, of course, as the only acromegalic in whom a basophilic adenoma was found. St. Rémy [30] and Bartlett [12] have fallen into the error of confusing α granules and mitochondria, because they used Altmann's acid fuchsin. The latter described α granules in the cells of a tumor of the hypophysis from a case not clinically acromegaly. The granules he saw were doubtless mitochondria.

Although some of our material was unfortunately fixed in Zenker's fluid or in Bensley's fluid,* both of which are unsuited to the hypophysis, most of the specimens were fixed in 10 per cent formalin, and this material, after being mordanted in 3 per cent potassium bichromate, is serviceable. All the specimens since 1920 were fixed in Regaud's fluid.

This report is based upon a study of the tissues removed at operation from thirty-five patients. This number includes all but

* It consists of equal parts of (1) saturate solution of mercury bichloride in 95 per cent alcohol and (2) 2.5 per cent aqueous solution of potassium bichromate. It is one of many formulae proposed by Bensley but is known in the pathological laboratory as Bensley's fluid and we shall continue to speak of it under this name.

three of the patients with acromegaly operated upon by Dr. Cushing at the Peter Bent Brigham Hospital. The material removed from two of the other three patients has been lost and that from the third was so imperfectly fixed that it was impossible to use it. In every case the diagnosis of acromegaly, or of dyspituitrism with acromegalic manifestations, was clearly established from the clinical examination alone, before the information obtained from microscopic examination.

As controls we have used the adenomas from fifty-four patients showing dyspituitrism without acromegalic manifestations. This includes all the cases operated upon in this clinic since 1920 from which the tissue removed was fixed in Regaud's fluid. (These statistics have been completed up to Nov. 1, 1924.)

Characteristics of Acromegalic Hypophysis

It might be well to state briefly at this point the results of our investigations, namely, the characteristics which distinguish the microscopic structure of the pars distalis in acromegaly from that of the normal hypophysis and from that of the adenomatous enlargement unaccompanied by acromegaly. These distinguishing characteristics may readily be appreciated from the series of figures in Plate XXXI.

In Fig. 1 the normal pars distalis may be seen to consist of columns of polygonal cells separated by delicate vascular sinuses. The cells consist of α and β chromophile cells, and chromophobe or reserve cells.

The pars distalis shown in Fig. 2 from a case of adenomatous enlargement *with* acromegaly is composed of a mass of rounded or polygonal cells, almost without blood supply. The cells are α cells containing α granules which are finer than normal and have a tendency to collect around the periphery of the cytoplasm. No β cells are present. Some of the cells may be without granules (Entgranuliertenzellen of Kraus). The size of the cells varies greatly, and multinucleated cells are numerous; some cells may have more than half a dozen nuclei. Amitotic nuclear divisions are commonly seen. Many cells are flattened onto the surface of adjacent cells in the form of a crescent (cf. Plate XXXII, Fig. 1).

The pars distalis in a case of adenomatous enlargement *without* acromegaly is shown by Fig. 3 to be composed of very thick columns

of cells separated by thin septa of connective tissue, carrying delicate capillaries. The cells are elongated, epithelial cells and are all chromophobe. Each cell has a simple elongated nucleus. The cells contain no other granules than mitochondria. The cell boundaries are often very indistinct (cf. Plate XXXII, Fig. 2).

Naturally all transitions are encountered between these three types of structure.

Case Reports

It is impossible and unnecessary to submit detailed reports of all the thirty-five patients. Four characteristic cases have been selected. Three of the patients were typical acromegalics, one having some evidence of gigantism; the fourth had a dyspituitary syndrome with marked acromegalic features. The tissues from these four cases were variously fixed — from the first in Zenker's fluid, from the second in formalin, from the third in Bensley's fluid and from the fourth in Regaud's fluid.

Realizing that many of the cases reported in the literature as acromegaly are doubtful examples of this disease, sufficient evidence will be presented, it is hoped, to establish the clinical diagnosis of our cases beyond question. Since the diagnosis of this disease depends so largely on bony changes and the external appearance of the patient, Roentgenograms and photographs are given, although the best photograph translates but imperfectly the impression one gets by direct observation, and in reproduction also many details are lost.

Case I

This patient entered the clinic at a time when it was the custom to fix in Zenker's fluid the tissues removed at operation.

Surg. No. 496. Acromegaly. Operation. Recovery.

October 16, 1913, Mr. C. M. W., an unmarried English solicitor, aged 24, was admitted, complaining of developmental changes and drowsiness.

The patient's father was 6 feet tall, but showed no disproportionate enlargement of the extremities. The patient had pertussis and scarlatina at 15, and pleurisy with effusion at 16. After recovering from this illness he began suddenly and rapidly to grow taller, so that in the 5 years between 16 and 21, his stature increased 8 inches. During the past 3 years he had grown little in height, but his hands and feet had enlarged markedly. Gradually over a period of 7 years, he had developed a host of symptoms such as failing memory, epiphora, polyphagia, constipation, hyperhydrosis, drowsiness, dull aching head pains, intermittent pains in the arms and legs, decreasing libido sexualis, failing vision, diplopia, definite subjective bitemporal hemianopsia, hallucinations of smell,

and marked protrusion of the lower jaw. The order of development of many of these symptoms is uncertain, but he had noticed the prognathism almost from the beginning.

On admission his height was 200 cm.; weight 108.6 kg. He was a strikingly tall, gigantic man, though listless in appearance and failing to make an impression of prowess corresponding to his stature. His skin was moist with perspiration and quite elastic. The hair on his head was fine in texture and luxuriant, but in other parts the growth of hair was scanty. All the bones of his skeleton were heavy and thick, especially the enlarged epiphyses. His arms and legs were particularly massive, with good muscular development. But the hands

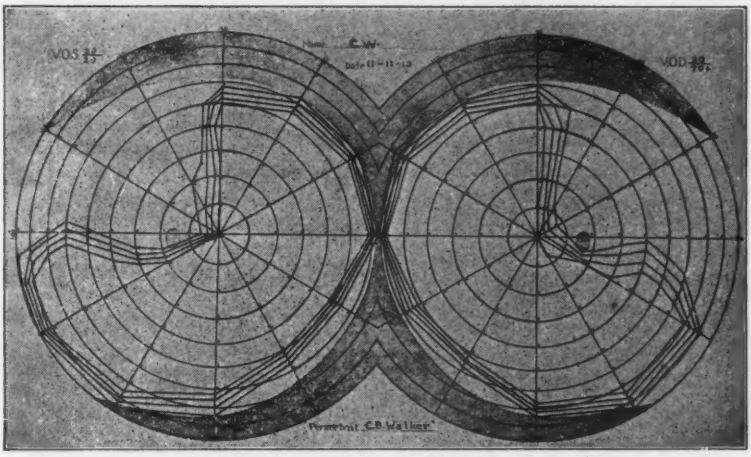

FIG. 1. Visual fields of patient I.

and feet were enormous. They were thick, soft, spadelike. The most marked enlargement of the head was shown in the frontal and facial bones. The eyes were wide apart and deeply set; the nares wide; the lips thick and heavy. The tongue was coarse and large; the teeth were widely spaced; the lower jaw was markedly prognathic (Plate XXXIII). The thyroid gland and genitalia were normal. The optic discs were pale and somewhat hazy.

His blood pressure was 120/74. Routine blood and urine examinations were normal. No metabolism determination was made. The visual fields showed characteristic defects in the upper two-thirds of both temporal regions (text Fig. 1). X-ray examination disclosed a moderate thickening of the frontal bone, an enlarged sella (Plate XXXV, Fig. 1), an overdeveloped occipital protuberance, an enormous frontal sinus, and marked hypertrophy of the mandible. The distal phalanges of both hands (Plate XXXIX, Fig. 1) and feet showed marked exostoses, and some tendency to bony overgrowth was apparent around the metatarso-phalangeal joints.

On October 20, 1913, Dr. Cushing exposed the floor of the sella turcica by the transphenoidal approach. The sellar floor was of dense bone which was chipped away with some difficulty and a smaller decompression than usual secured. A

crucial incision was made in the exposed dural capsule, and a few fragments of the adenoma were removed.

He recovered promptly from the operation and was discharged November 5, 1913, with some early improvement in his visual fields. He subsequently saw service with the British Army in France and on May 6, 1922, last wrote that his malady appeared to be stationary.

Histological examination of tissue removed at operation

The tissue was fixed in Zenker's fluid and stained with hematoxylin and eosin. As no fresh sections were available, one of the original sections was restained with neutral ethylviolet-orange G (Plate XL, Fig. 1).

The tissue consists of polygonal tumor-cells with very few blood vessels and scanty connective tissue. The cells vary greatly in size. They have distinct cell-boundaries and large vesicular nuclei, several of which are often contained in one cell. Crescent formation may be seen.

Many cells contain α granules tending to congregate in the periphery of the cytoplasm. Because of the imperfect method of preparation it is impossible to determine whether all the cells possess this granulation, but it is obvious that most of them have some α granules. β granules were not found.

The tissues from twenty-two other patients have been fixed in Zenker's fluid or in formol-Zenker. This fixation is not the best, in our opinion, but tissues so fixed may be used as will be shown. In the following case formalin was used as a fixative.

Case II

Surg. No. 826. Dyspituitrism with marked acromegalic manifestations. Operation. Death from meningitis. Necropsy.

January 23, 1914. Mr. J. H. D., a school teacher of 46, was admitted complaining of skeletal overgrowth, adiposity, drowsiness and failing vision. Referred by Dr. J. W. Courtney of Boston, Mass.

The patient's father and 8 siblings were of unusual stature. He had been married for 21 years and had 9 children, the youngest being 4 years of age. He had always been healthy. He reached a height of 5 feet 10 inches at the age of 22, and weighed 170 lbs. He maintained this height and weight until his present illness.

The onset of his present illness was gradual. He noticed a gradual gain in weight and increasing size of his hands, feet and features 8 years before. About a year later he began to feel drowsy and became markedly constipated. About 4 years before admission his memory became poor and his speech indistinct. At the age of 44 he noticed rather suddenly that vision in his right eye was imperfect. During the past year his left eye became similarly affected. Beginning about two years ago his libido sexualis diminished until at admission it was entirely absent.

Examination showed a large man whose height was 179 cm. and weight 110 kg. He was drowsy; he had a defective memory; and his speech was indistinct because of his large tongue. The skin was coarse and elastic over the extremities; the palms and soles were quite moist. Numerous fibromata mollusca were present over the neck and chest. The hair was normally abundant and coarse,

but pubic hair showed a feminine distribution. The extremities were characteristic of a markedly advanced acromegalic state (Plate XXXIV). There was no prognathism. The optic discs were pale, with sharp outlines. The thyroid gland was not palpably enlarged. Blood pressure was 132 mm. Hg.

The X-ray disclosed a ballooned-out sella turcica with erosion of its bony walls (Plate XXXV, Fig. 2). The terminal phalanges showed marked tufting (Plate XXXIX, Fig. 2). A left homonymous defect, more advanced in the right eye, was present in the visual fields (text Fig. 2). His temperature while under observation tended to be subnormal and there was a slight polyuria but no glycosuria. The blood Wassermann reaction, routine blood and urine exam-

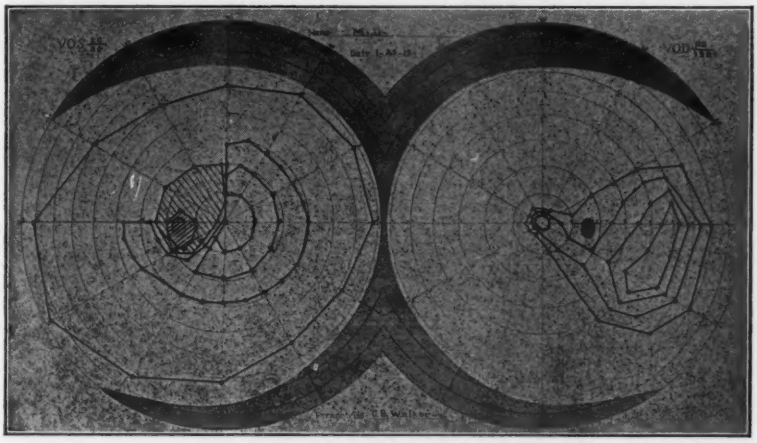

FIG. 2. Visual fields of patient II.

inations were negative. He left the hospital after 5 days' observation to return again the 21st of February, 1916 (Surg. No. 4310).

During the interval he had gained in weight 5.5 kg. His vision had decreased perceptibly, and examination of the visual fields showed an increase in the field defects. His basal metabolism was now examined for the first time and was found to be + 22.

On March 3, 1916, Dr. Cushing did a transphenoidal operation and found that the tumor had already completely eroded the floor of the sella and extended into the sphenoidal sinuses. The thin and distended capsule of the tumor was incised and the soft growth extruded itself in large masses. More material was removed with the pituitary spoon. The tumor was somewhat more vascular than usual.

The patient recovered well from the immediate effects of the operation, but five days later his temperature suddenly rose to 104.2° and in a few hours he became rapidly comatose and died.

A necropsy limited to the head was performed 7¾ hours post-mortem. A large tumor mass occupied the region of the sella turcica. Projecting from its lower surface was an additional mass of tumor which had herniated through the

operative incision of the capsule. The carotid arteries were widely separated by the tumor, and the optic nerves were angulated by a band of dura and greatly flattened by the upward pressure from the growth, which extended upward for about 3.5 cm. above the sellar floor. The tumor mass was rather firm and quite black (after formalin fixation), suggesting hemorrhagic infiltration. There was no gross evidence of meningitis.

Histological examination of tissues removed at operation

Tissue fixed in 10 per cent formalin and mordanted in 3 per cent potassium bichromate for 8 days, stained with neutral ethylviolet-orange G, acid violet-eosin, and hematoxylin and eosin. The tumor consists of a structureless mass of polygonal cells and a very few thin-walled blood vessels. The cells and nuclei vary greatly in size. The cytoplasm is rather scanty and only a few cells with more than one nucleus are seen. Many of the cells contain clear-cut α granules, usually disposed in the periphery of the cell (Plate XL, Fig. 2). No β granules are present.

Histological examination of tumor removed post-mortem

Three blocks were cut from the formalin-fixed tumor — one from the sellar contents, and two from the upper portion. They were mordanted for 8 days in 3 per cent potassium bichromate solution, cut, and stained with neutral ethyl-violet-orange G, acid fuchsin-methyl green, acid fuchsin-acid violet, and hematoxylin and eosin.

The structure is largely similar to that found in the tissues removed at operation, except that a larger proportion of the cells contain α granules.

An attempt to find a more suitable fixative was made by introducing a formula proposed by Bensley, and the tissues from the following patient were fixed in this way.

Case III.

Surg. No. 1664. Acromegaly. Operation. Recovery.

August 19, 1914, M. J. C., an unmarried school teacher of 25, was admitted complaining of headaches, drowsiness, overgrowth and eye trouble. Referred by Dr. J. B. Mason of London, Ky.

He came of a family of tall people. His father was 6 feet tall. His paternal great uncle was 7 feet 1½ inches tall and was exhibited in a traveling circus under the title of the "Kentucky giant."

The patient had typhoid fever at the age of 7 years. His secondary sex characters appeared at the age of 14. When 16 years of age he first noticed a prominence of the forehead, and an increase in the size of his hands and feet and lower jaw. At 19 he suffered from severe night sweats, which still continue. At 20 he began to have severe, dull, occipital headaches; during one period it seemed as though he were losing his eyesight, but this subsequently improved. Three to four years before admission he noticed a markedly increased hunger with especial appetite for sweets, increased thirst, and increased urinary output. For the past two years he had been somewhat somnolent, even in the daytime, and had become fatigued very easily. His weight at 17 was 180 pounds; at 19, 190; at 20, 212; and at admission 197 pounds.

Examination showed a tall, heavy man of strikingly acromegalic type (Plate XXXVI). His height was 175.5 cm.; weight 90 kg. The skin was somewhat sallow, moist, very elastic, and several pigmented moles were present over the body. The hair of the scalp and beard was coarse and heavy, but normal everywhere else. The thyroid gland and external genitalia showed no evident abnormalities. There was slight bilateral primary atrophy of the optic discs.

His blood pressure was 155/95. X-ray films of the skull showed marked prognathism and enormous frontal sinuses. The sella turcica was much enlarged, its floor depressed, and the dorsum sellae very thin (Plate XXXVIII, Fig. 1). Films of the hands showed marked terminal "tufting" of the phalanges

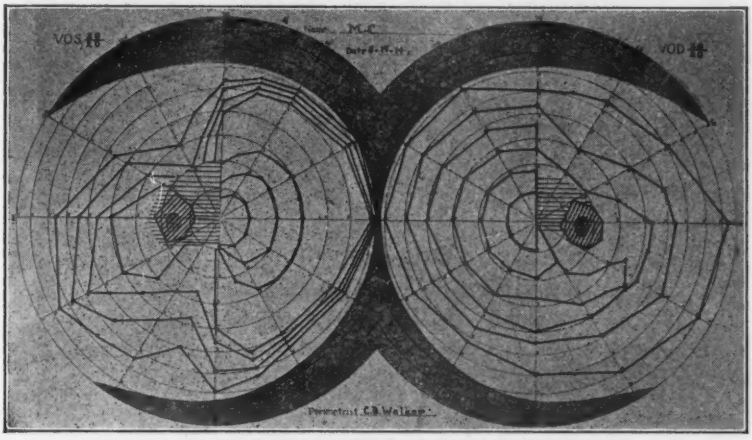

FIG. 3. Visual fields of patient III.

(Plate XXXIX, Fig. 3). There seemed to be no remaining evidence of the epiphyseal cartilages. Visual fields showed bitemporal defect with 20/20 vision (text Fig. 3). His temperature was normal and there was no polyuria. Marked hyperhidrosis was present.

Blood, urine and Wassermann tests were negative. His alimentary sugar tolerance was over 250 grams of glucose. No metabolism estimation was made.

On August 22, 1914, Dr. Cushing exposed the sella turcica by a transphenoidal approach. When the dural capsule over the tumor was incised, soft yellowish tumor-tissue extruded itself through the opening. A considerable amount of this tissue was spooned away.

He recovered rapidly and was discharged August 29, 1914. His headaches were relieved and slight improvement had occurred in central vision. He reported on April 21, 1922, that his condition was not markedly changed except that his lower jaw was perceptibly larger. Though his vision is retained, he has been obliged to give up his teaching work.

Histological examination of tissue removed at operation

Tissue fixed in Bensley's fluid and stained with neutral ethylviolet-orange G, acid violet-eosin, and hematoxylin and eosin.

The tumor consists of a structureless mass of cells with practically no supporting tissue except in the thin walls of the rare blood capillaries. The cells have very definite cell-boundaries and vary greatly in size and shape. The nuclei are vesicular and many cells contain from two to six of them. Crescent formation is occasionally seen.

The cytoplasm of these cells is packed with α granules (Plate XL, Fig. 3). No β granules are to be found.

Undoubtedly the best fixative for the hypophysis is Regaud's fluid, and since 1920 this fixative has been employed as a routine for all hypophysial adenomas. The following case was chosen because it is an example of early and slight acromegaly. It is even doubtful whether one could be sure of the diagnosis from the photographs given here. Yet there was no doubt of the diagnosis in the minds of any of us who saw the patient in this clinic, and microscopic examination of the tissues removed at operation showed a typical picture of what we have described here as acromegalic adenoma.

Case IV

Surg. No. 22532. Acromegaly. Operation. Recovery.

November 4, 1924, admission of Vivian H., a clerk, 22 years of age, complaining of headache, failing vision, and enlargement of the hands and feet. Referred by Dr. R. T. Woodyatt of Chicago, Ill.

There was no history of unusual stature or of any other developmental anomalies in the family. The patient had never suffered from any serious illness. Her menses began at the age of 13, and were always regular until November, 1923. Since that date they were irregular and scanty until April, 1924, when they ceased completely.

The present illness began about two years before admission to the hospital, with a feeling of discomfort over the left eye and occasional slight bitemporal headache. The headache had steadily increased in frequency and was now present practically every day. Accompanying the headache would occasionally appear oedema, swelling and pain around the left eye. One year before her menses became irregular, and she noted that she had to wear larger gloves. She had noticed no change in her features. Profuse perspiration had annoyed her for the last six or eight months. Her vision began to fail in June. In the last 2 years she had gained about 12 pounds in weight.

The patient had been given a series of 7 Roentgen-ray treatments since July, 1924, but her vision continued to fail, so she was sent to this clinic.

She was a strong, active young woman; not at all obese. There was some coarseness of the nose and lips which the patient herself had not noticed. It is scarcely noticeable in the photograph (Plate XXXVII), but comparison with photographs taken four years earlier leaves no doubt of its presence. There was no prognathism. The hands were typically acromegalic, having thick, square fingers and moist palms. There was little deformity of the feet. Roentgenographs show an enlarged sella turcica (Plate XXXVIII, Fig. 2). There was no

"tufting" of the terminal phalanges of the fingers (Plate XXXIX, Fig. 4). The skin was soft and moist. The hair was normal in texture and distribution.

There was an early bitemporal defect in the visual fields (text Fig. 4). Temperature and pulse rates were normal. Urinary output was not increased. The urine contained no sugar. The basal metabolism was −7. Her height was 167 cm., and her weight 57.7 kg.

On November 12, 1924 Dr. Cushing did a transphenoidal operation. When the dura underneath the tumor was incised, soft neoplastic tissue extruded itself in large masses. Additional tissue was removed with the usual instruments.

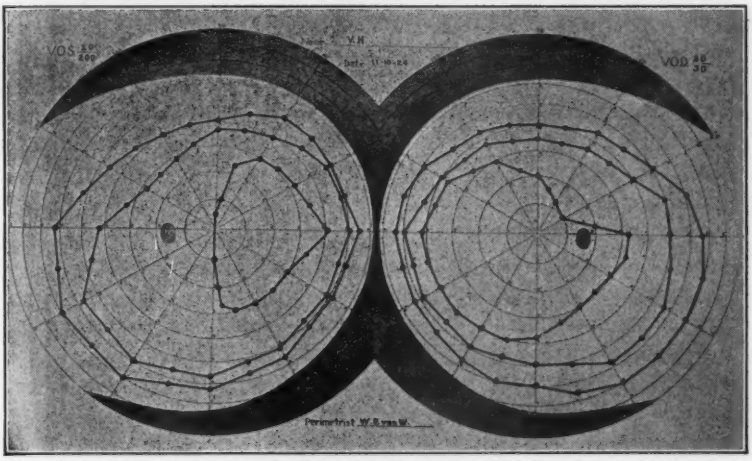

FIG. 4. Visual fields of patient IV.

The patient recovered promptly and was discharged on December 1, 1924. Her visual fields had rounded out normally and visual acuity was much improved.

Histological examination of tissues removed at operation

The tissues were fixed in Regaud's and in Zenker's fluids and were stained with hematoxylin and eosin, neutral ethylviolet-orange G, and with acid violet-eosin.

The tissue is composed of a mass of polygonal cells, with very little connective tissue except that around rare vascular sinuses. Many multinucleated cells are seen. The great majority of the cells contain α granules. Many of the cells are so packed with these granules as to appear almost solidly blue (Plate XL, Fig. 4).

SUMMARY OF RESULTS IN ALL CASES

The four cases given in detail are typical of our material, both from the clinical and pathological points of view. The effects of the various fixatives used are clearly illustrated.

The tissues removed from twenty-three patients were fixed in Zenker's fluid or formol-Zenker. This fixation has the defect of agglomerating the α granules and also causes artificial granulation of the cytoplasm. The chromatin granules also stain intensely after this fixation, and the α granules are not differentiated clearly from the background (cf. Plate XL, Fig. 1). The color contrast may be heightened by mordanting the tissue in 3 per cent potassium bichromate for some days before embedding it. Paraffin sections may also be so treated. In this way we have been able to demonstrate numerous α cells in 19 of the 23 cases. In 4 cases we could not find α cells. In 3 of these (Surg. Nos. 4102, 6037, 4430) the cells contained coarse granules, but we were unable to obtain a sufficiently distinct differentiation to be sure they were α granules. However, they were not β granules. Two of these patients were frank acromegalics; the other had a dyspituitary syndrome with some acromegalic features. The fourth tumor in which we could not find granules came from a patient (Surg. No. 10223) who was not diagnosed as acromegaly at the time of operation, but returned later with characteristic changes and died in diabetic coma. Permission for a necropsy could unfortunately not be obtained. The general structure of the tumor in each of these four cases, the loose arrangement of the cells, absence of connective tissue septa, multinucleated cells, etc., made us feel that we were dealing with the same type of adenoma as in the other cases.

In two cases, only formalin-fixed tissues were available for examination. This material is quite suitable, but preparations made from it do not photograph well, as the contrast is not great (cf. Plate XL, Fig. 2). However, in each of these two cases numerous α cells were found. The hypophyses from four necropsies done on acromegalic patients were also examined. They were all fixed in formalin and all contained great numbers of α cells.

There was only one case in which the tissues removed at operation had been fixed in Bensley's fluid alone. This fixation causes granulation of the cytoplasm so that the preparations photographed badly (cf. Plate XL, Fig. 3). Under the microscope the distinction between the artificial granulation of the cytoplasm and the α granules is easier, for the latter are stained blue with neutral ethylviolet, while the former is orange. The contrast, however, is not so sharp as could be wished and the fixative is on the whole quite unsatis-

factory. However, it shows unmistakably a preponderance of α cells. The tissues from the last 9 patients were fixed in Regaud's fluid and in each one great numbers of cells were found so full of granules in many instances that in the microphotograph they appear as a black mass (cf. Plate XXXI, Fig. 2, right).

In contrast to the thirty-five acromegalic hypophyses there are fifty-four hypophysial adenomas taken from patients who showed no signs of acromegaly. In none of them could we find more than a few α cells, scattered through many sections, after a long search. In all but three or four of them no α cells could be found. The general structure of these chromophobe tumors was also different from that of the acromegalic tumors as we have already explained.

Cases from Literature

The following are the cases of acromegaly reported in the literature in which sufficient details of the histological technique are given to enable one to be sure that the technique was adequate for the demonstration of the α granules. We have noted every case in which, in our opinion, the author has shown α granules, although, of course, they were not called by this name.

I. Fränkel, Stadelmann, and Benda,[3] 1901.

Case I. Tumor of hypophysis extending up into third ventricle. Non-granular cells predominated, but many α cells were seen.

Case II. Tumor of hypophysis eroded sellar floor and filled sphenoidal cells. Cells contain fairly coarse α granules.

Case III. Large pituitary tumor — composed almost entirely of α cells.

Case IV. Fairly normal appearing acini around periphery, gradually going over into irregular mass of tumor cells, which are practically uniformly α cells, stuffed with granules. (Presumably toluidin blue-sodium sulphalizerinate used in all cases.)

II. Launois et Roy,[21] 1903.

Large tumor extending into right cerebral hemisphere. Cells of tumor packed closely together are of variable shapes. Many have 2, 3, even 7 nuclei. Amitotic division of nuclei

common. Nearly all cells eosinophile. No cyanophiles. (Presumably used Launois' methylviolet method.)

III. Huchard et Launois,[16] 1903.

Sella turcica 27 mm. × 19 mm. Pituitary small (0.80 gm.); almost lost in the big sella. Necropsy done 2 days post-mortem. Marked connective tissue sclerosis. Authors note delayed post-mortem, and unsatisfactory nature of material. Say most of the cells seem to be cyanophile. (Used presumably Launois' method with methylviolet. Not mentioned in text.) Since this method is a specific stain for α granules, the adenoma was undoubtedly eosinophilic.

IV. Cagnetto,[13a] 1904.

Case II. Hypophysial tumor size of "hen's egg," with metastatic deposit along spinal cord. In the periphery of the tumor fairly well-defined acini. Center of tumor consisted of an irregular mass of cells with no definite arrangement. In the periphery and for a considerable distance into the tumor all the cells were α cells. Fixed in Müller's fluid and used Benda's method and methyl violet. Metastases composed of chromophobe cells.

V. Ballet and Laignel-Lavastine,[26] 1905.

Hypophysis weighed 0.75 gram. There was a great increase in the interalveolar tissue. The glandular cells were almost entirely α cells. No β cells were seen. Iron hematoxylin of Heidenhain.

VI. Lewis,[19] 1905.

Hypophysis said to be macroscopically normal. The component cells very irregularly grouped in the intercapillary spaces with very little connective tissue. A few chromophobe and basophile cells found along boundary between ant. and post. lobe. All the rest were α cells. Some of them contained 2 or 3 nuclei. Used among other methods, iron hematoxylin on formalin-fixed material.

VII. Cagnetto,[13b] 1907.

Case I. Hypophysial tumor weighed 8.55 grams. In the periphery of the tumor typical acini. The center of the growth consists of larger cells not definitely arranged along

vessel walls. Notes many giant cells with 6 or 7 nuclei. States that all the cells of the tumor are chromophobe. Technique not noted, but probably Benda's method and acid fuchsin. Necropsy 30 hours post-mortem.

Case II. Cystic tumor of hypophysis. Necropsy 29 hours post-mortem. Fragments of growth around wall of cyst composed of both α and chief cells. Used Benda's method.

VIII. Alquier and Schmiergeld,[22] 1907.

Case I. Hypophysis weighed 8 grams. The hyperplasia composed almost entirely of α cells, with large vesicular nuclei. Used iron hematoxylin of Heidenhain. A few basophiles were seen (?) and rarely chromophobe cells. The cells were crowded against each other without any regular disposition. A few blood vessels and almost no connective tissue.

IX. Stumme,[7] 1908. Operative Specimen.

Müller-formol. Used iron hematoxylin, Mallory (?) and Kresofuchsin. Composed of small lymphocyte-like and spindle-shaped cells not arranged in alveoli. Many of them contained numerous fine granules not staining distinctly.

X. Erdheim,[9] 1909.

Tumor in sphenoid sinus. Cells filled with α granules. (Heidenhain's iron hematoxylin.) Hypophysis looked normal (19 × 12 × 6 mm.).

XI. Wurmbrand,[33] 1909–10.

Case II. Operative Specimen.

Used iron hematoxylin among other stains. Cells in an irregular mass with practically no struma. Filled with fine α granules.

Case III. Operative Specimen.

Used iron hematoxylin among other methods. Mass of cells with no connective tissue stroma. Nuclei vary enormously in size. Many cells with 3–5 nuclei. In the cytoplasm of the cells are numerous α granules, finer and more sparsely distributed than in the normal cells.

XII. Lewis,[20] 1910.

Material removed at operation. Fixed in Zenker's fluid and stained with neutral gentian, etc. Material shows enlarged

acini with little interacinous connective tissue. Many of the cells in karyokinesis. Large majority contain α granules.

XIII. Erdheim,[30] 1910.

Case II. Hypophysis measured 1.5 × 1.4 × 0.95 cm. Adenoma (11 × 6 mm.) in pars distalis composed of α cells. Used iron hematoxylin of Heidenhain. The growth was composed of a disorderly mass of cells with large vesicular nuclei. There was very little connective tissue.

XIV. Ballet and Laignel-Lavastine,[11] 1912.

Hypophysis weighed 0.75 gram. There was a great increase in the interalveolar tissue. The glandular cells were almost entirely α cells. No β cells were seen. Stains used for α cells not mentioned, but refer to previous case (V) where they used iron hematoxylin of Heidenhain.

XV. Leotta,[18] 1912.

Tumor of hypophysis 4 × 3 cm. Composed of loose mass of rounded cells with large vesicular nuclei. Few blood vessels. Many cells have more than one nucleus. As many as 7 in a single cell. Most of the cells are eosinophilic. Tissue fixed in 10 per cent formalin and stained with Heidenhain's iron hematoxylin.

XVI. Reinhardt and Creutzfelt,[24] 1913.

Tumor of hypophysis 2 × 3 cm. Very little stroma. Cells loosely arranged with no order. Very fine granules in cells. From 2–8 nuclei seen in a single cell. Used iron hematoxylin and Kraus' hematoxylin.

XVII. Kraus,[17] 1914.

Case 24. Hypophysis a tumor size of a "cherry." Some normal anterior lobe tissue in the periphery. Tumor consists of rounded cells varying in size. Many of them are α cells. The α granules are not so marked as in the normal cells. Very little connective tissue struma and few blood vessels. Evidently used Kraus' hematoxylin.

Case 25. Hypophysis 18 × 20 × 13 mm. A typical eosinophilic adenoma with very little normal anterior or posterior lobe. Kraus' hematoxylin used.

Analysis of the Literature

In only two cases, to our knowledge, where presumably adequate technique was used, has the tumor been stated not to be an eosinophilic adenoma. The first was Cagnetto's Case I.[13b] We need only indicate that the necropsy was done 30 hours post-mortem, giving ample time for the delicate granulation to be lost. No such objection can be brought against Stumme.[7] His material was removed at operation and presumably fixed at once. It was fixed in Müller's fluid and properly stained. His observation is, therefore, probably correct that the cells contained a fine granulation which stained poorly by methods for α granules. Such a granulation we have seen in the case of a fat patient with dyspituitrism and some acromegalic features. It is unfortunate that Stumme gives sketches of his patient instead of photographs, for in sketches interesting details, such as texture of skin, etc., cannot be judged.

Cagnetto, when he thought he had found a case of acromegaly in which the tumor was not eosinophilic, stated that now there was needed, to overthrow the hyperpituitary theory of acromegaly, only a case of tumor in which the cells contained α granules but the patient was not acromegalic. Such a case he thought was supplied by Zak.[8] But Zak, who evidently is familiar with the literature, and gives a very judicious estimate of his case of pituitary tumor without acromegaly, makes no claim to have discovered an eosinophilic adenoma here. He simply states that "the demonstration of Benda's secretory granules, because of the unfavorable fixative (alcohol) for this purpose, met with only indifferent success."

It is repeatedly noted in the above reports that many of the cells in the hypophysis of acromegaly contain several nuclei. The frequence of amitotic division is also noted. These phenomena have been long known and were seen by Osborne (1922),[22] Neal and Shattuck (1898),[21] Brooks (1898),[34] Dalton (1898),[15] Claude and Baudouin (1911),[14] Meyer (1913),[30] Ausch (1918),[10] and many others.

In three patients often quoted in the literature as acromegalic[38] the pituitary body is said to have been normal (Bonardi,[4] Petren,[23] Yamada[35]). In the cases of Petren and Yamada we are willing to admit that the hypophysis was normal, but question whether either patient was acromegalic. The hypophysis from Bonardi's patient

was four times normal weight (2.975 gms.), and details of the histological examination given seem to indicate that it was not adequate, a fatal error in the absence of a tumor, as the cases of Lewis and of Huchard and Launois demonstrate. Besides, as a case of Erdheim (X) shows, when the pituitary body is of normal size, a search must be made of the sphenoid region and pharynx.

We must, finally, mention the often quoted report of Bleibtreu [6] in which a hemorrhagic cyst was found in the hypophysis of a giant acromegalic. There is no evidence whatever from the history to determine when the hemorrhage took place, and we feel no hesitation in affirming that it occurred in a soft adenoma, after the acromegalic syndrome was established. Such a hemorrhage we have ourselves observed.

It is obvious, therefore, that, in the light of our own experience and after a careful scrutiny of the literature, we believe that satisfactory evidence does not exist of the presence of acromegaly without an increase in the number of the eosinophilic cells of the pars distalis of the hypophysis cerebri.

Conclusion

In view of the foregoing description of histological material and analysis of the literature, we feel justified in concluding that, in those cases of acromegaly at least in which the gland reaches a size sufficient to demand operation, a constant pathological change takes place in the pars distalis of the hypophysis cerebri, consisting essentially of an adenomatous formation composed of α cells. The normal acinous structure tends to be lost; the cells lie in a loose mass, practically without stroma; the nuclei vary greatly in size and as many as half a dozen may be present in one cell; the cells contain α granules which are finer than the normal α granules and which tend to collect in the periphery of the cytoplasm.

This study was aided by the Philip Gray Fund for the study of brain tumors, at the disposal of Dr. Harvey Cushing.

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DESCRIPTIONS OF PLATES XXXI–XL

PLATE XXXI

Fig. 1. Microphotographs of normal hypophysis cerebri.
left. Hematoxylin and eosin stain. x 300.
right. Acid fuchsin-acid violet stain. x 850.
a. α cells. b. β cells.
c. chromophobe cells.

Fig. 2. Microphotographs of chromophile adenoma from a patient with acromegaly.
left. Hematoxylin and eosin stain. x 300.
Note absence of connective tissue and of blood vessels; also multinucleated cells.
right. Neutral ethylviolet-orange G stain. x 850.
Cells are packed with α granules, which alone appear black in photograph.

Fig. 3. Microphotographs of chromophobe adenoma of hypophysis from a patient with hypopituitary syndrome.
left. Hematoxylin and eosin stain. x 300.
Note the elongated epithelial cells in large masses, separated by thin connective tissue septa carrying small blood vessels.
right. Phosphotungstic acid-hematoxylin stain for mitochondria. x 850. The fine granular mitochondria are so numerous that they outline well the boundaries of the cells.
No other granules than mitochondria exist. These may persist in the cells and be mistaken for α granules.

PLATE XXXII

Fig. 1. Microphotograph of hypophysial adenoma from a patient with acromegaly.
Fixed in Zenker's fluid.
Hematoxylin and eosin stain. x 850.
Note multinucleated cells and crescent formation.

Fig. 2. Microphotograph of hypophysial adenoma from a patient with a hypopituitary syndrome.
Fixed in Zenker's fluid.
Hematoxylin and eosin stain. x 850.
Note elongated cells along the septa and the indistinct cell boundaries.

PLATE XXXIII

Case I. Note the prognathism and prominent frontal sinus.

PLATE XXXIV

Case II. Note the adiposity, coarsening of features and extremities, and absence of prognathism.

PLATE XXXV

Fig. 1. Lateral roentgenograph of sella of Case I. Nat. size.

Fig. 2. Lateral roentgenograph of sella of Case II. Nat. size.

PLATE XXXVI

Case III. Note prognathism and prominent frontal sinus.

PLATE XXXVII

Case IV. Note slight coarseness of nose and lips and absence of prognathism, also thick, square fingers.

PLATE XXXVIII

Fig. 1. Lateral roentgenograph of sella of Case III. Nat. size.

Fig. 2. Lateral roentgenograph of sella of Case IV. Nat. size.

PLATE XXXIX

Roentgenographs of middle fingers of Cases I, II, III, IV.
Note width of fingers, and tufting of terminal phalanges in every case except the last.

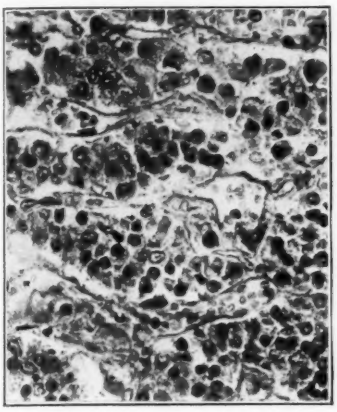
PLATE XL

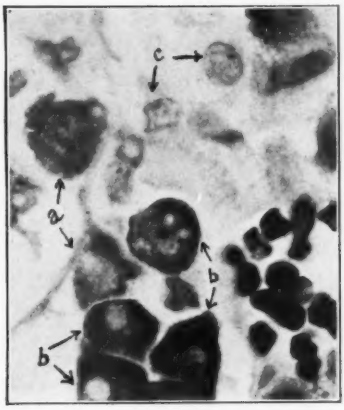
Fig. 1. Microphotograph of tumor from Case I. Zenker fixation.
Neutral ethylviolet-orange G stain. x 850.
Note that the nuclei are well stained but α granules not well differentiated.

Fig. 2. Microphotograph of tumor from Case II. Formalin fixation.
Neutral ethylviolet-orange G stain. x 850.
Note that the α granules are well differentiated but contrast is not great.

Fig. 3. Microphotograph of tumor from Case III. Bensley fixation. Neutral ethylviolet-orange G stain. x 850. Note the artificial granulation of the protoplasm due to the fixative. Under the microscope the α granules may be distinguished by their blue color. The differentiation is not so distinct as it is on Regaud-fixed material.

Fig. 4. Microphotograph of tumor from Case IV. Regaud fixation. Neutral ethylviolet-orange G stain. x 850. Only the α granules and erythrocytes appear black in photograph.





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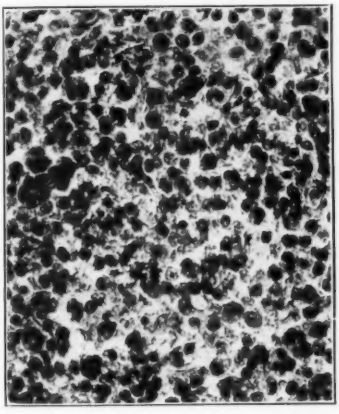

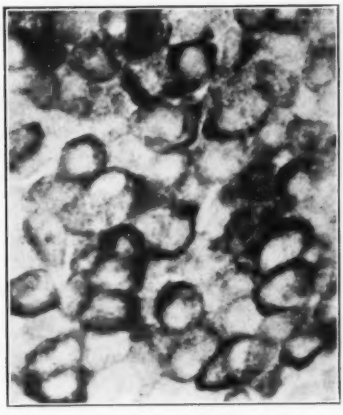

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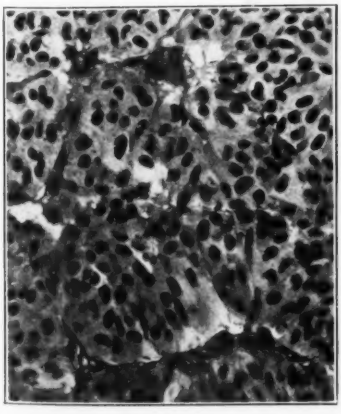

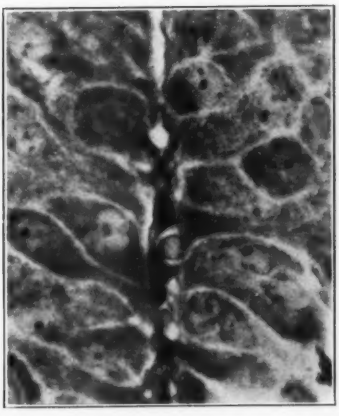

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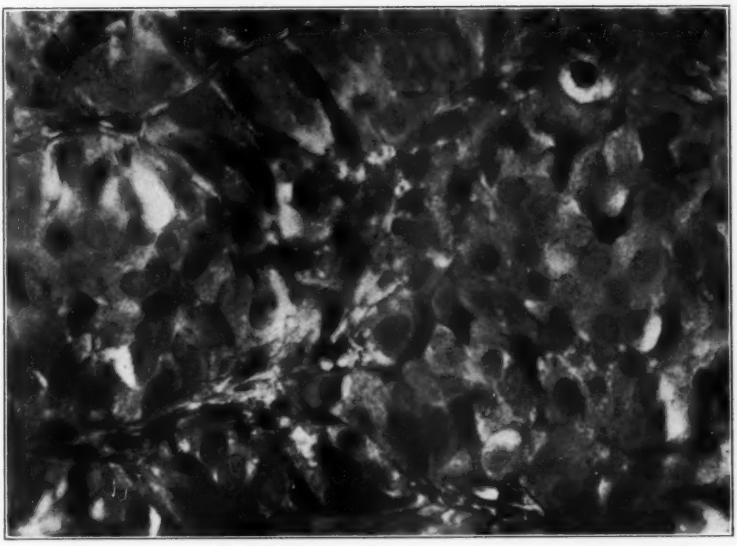
Bailey and Davidoff Hypophysis cerebri in acromegaly



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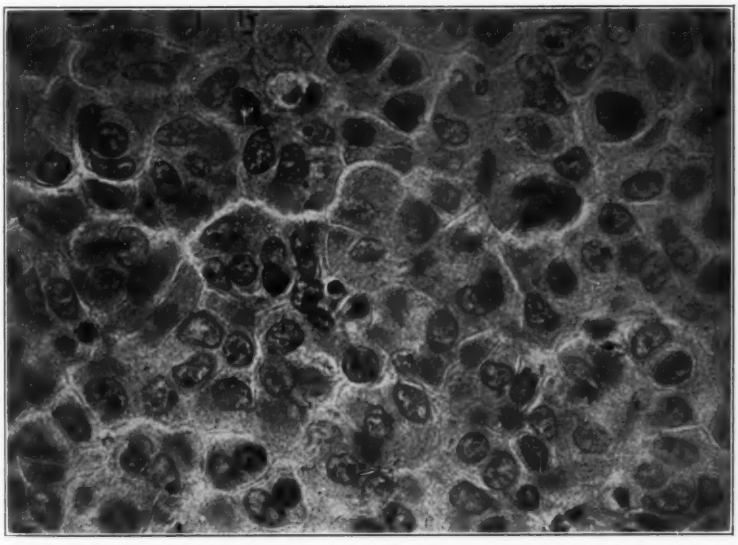

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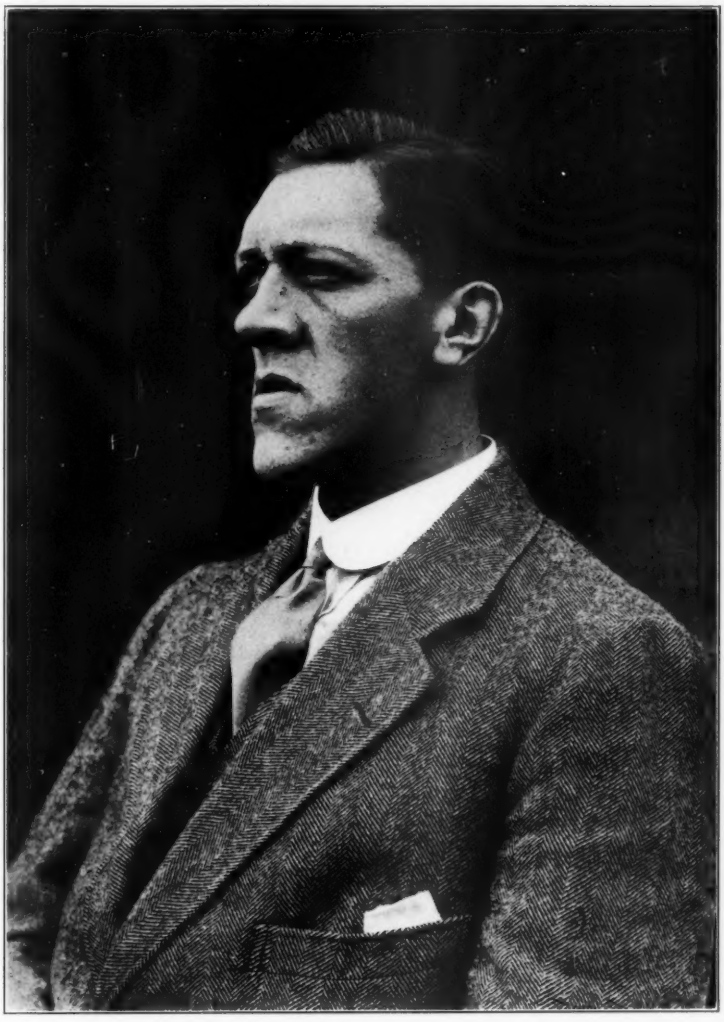
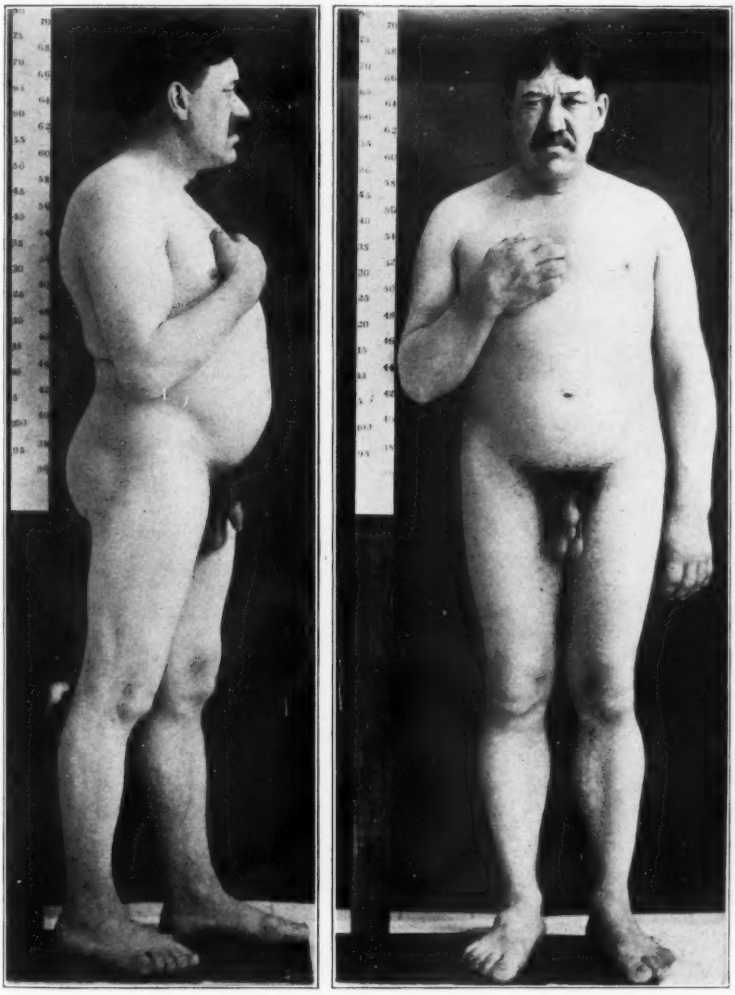


Bailey and Davidoff Hypophysis cerebri in acromegaly



Bailey and Davidoff Hypophysis cerebri in acromegaly

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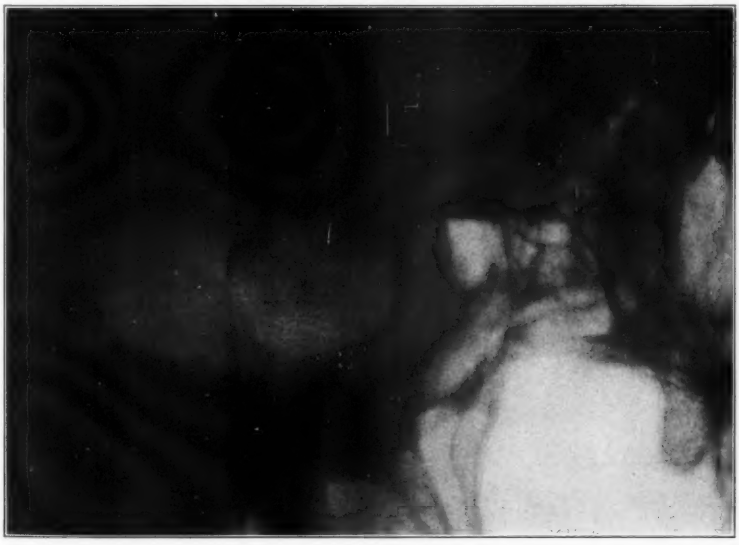

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Bailey and Davidoff Hypophysis cerebri in acromegaly

Bailey and Davidoff

Hypophysis cerebri in acromegaly

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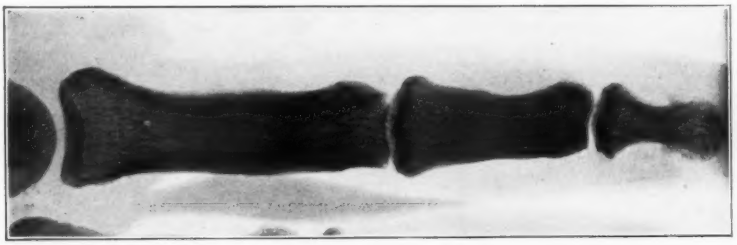
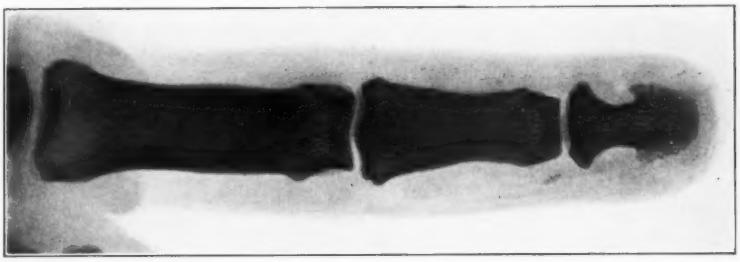
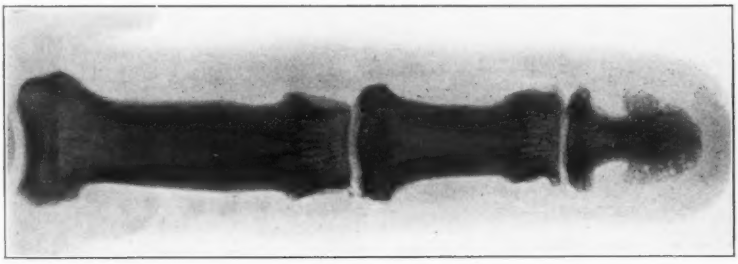
Bailey and Davidoff Hypophysis cerebri in acromegaly







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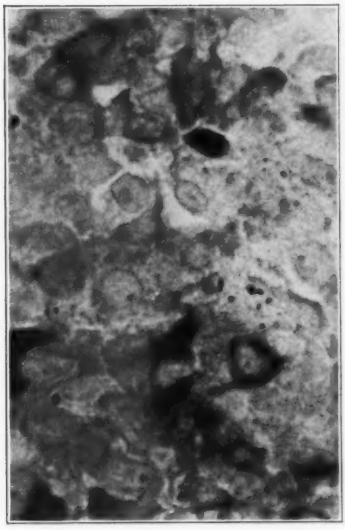

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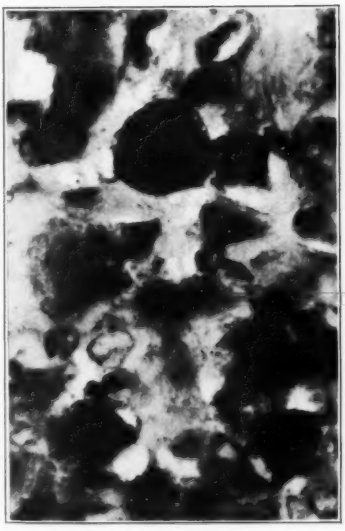

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Bailey and Davidoff

Hypophysis cerebri in acromegaly

THE PATHOLOGY OF THE HYPOPHYSIS *

I. THE PRESENCE OF ABNORMAL CELLS IN THE POSTERIOR LOBE

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The deficiency of accurate knowledge as to the function or functions of the hypophysis, and the effect on the general economy of the body of pathologic processes in this organ, render it desirable to seek information concerning its pathology from all possible sources. This small gland is all too frequently neglected in the usual post-mortem examinations even when the head is opened and the brain removed. It has been our practice for some years to examine in serial sections all hypophyses removed at necropsies. As a result of these routine examinations a number of distinct abnormalities have been observed. It is proposed in a series of papers, of which this is the first, (1) to describe the various lesions encountered; (2) to search in the associated pathology for some clew as to the etiology of the pathologic process in the hypophysis; and (3) to make a careful analysis of the clinical histories for possible clinical manifestations of the lesion.

In 1922, one of us[1] described the presence of a group of abnormal cells in the posterior lobe of the hypophysis. Since that time, eight similar cases have come under our observation. Two papers dealing with a like condition have appeared in the literature.

Sternberg[2] has described in the neurohypophysis a group of abnormal cells which he designated as a Choristoma. The patient was a man 65 years old. The outstanding clinical manifestations were fatigue on slight exertion, without dyspnoea or palpitation; marked anemia without emaciation; a generalized persistent and obstinate edema that was neither cardiac nor renal in origin; and a marked retention of water and sodium chloride. At necropsy a large medullary carcinoma of the stomach was found. The heart, kidneys, thyroid, lungs, liver and brain showed no gross pathologic changes. The hypophysis was not enlarged. On surfaces made by sectioning there was seen in the posterior lobe a very small white

* Received for publication December 22, 1924.

mass, distinctly visible against the general grayish brown color of the lobe. On microscopic examination this mass was found to be composed of cells, in all essentials of morphology and arrangement, similar to those shown in Figure 1 of our series.

In 1922, Priesel [3] reported 29 cases in which he found very small masses of misplaced tissue in the posterior lobe of the hypophysis. In some five of his cases the cells were distinctly more spindle-shaped and were not, therefore, in all respects similar to the instances reported by one of us and by Sternberg. Very scant clinical data were given by Priesel. None of his patients showed edema that could not be readily accounted for either on a cardiopathic or a nephropathic basis.

The groups of abnormal cells in our cases varied somewhat in size, but were essentially similar in morphology and arrangement. A general description of these masses of cells will be given after briefly summarizing the other features of the cases.

Case 1. Colored male. Estimated age, 65 years. Found dead (murdered) in a basement. No clinical history was obtainable. He was six feet, two inches tall and "rawboned" but not emaciated; the hands and feet were quite large; prognathism was not present. There was no abnormal growth of hair on the body. Adrenals, thyroid and and testicles showed no important gross pathologic changes, and were of approximately normal size. The hypophysis weighed 0.65 gram. In the posterio-lateral quadrant of the neurohypophysis, about 0.5 mm. from the median line, was a mass of abnormal cells measuring approximately 0.75 mm. in diameter. Cells from the middle lobe had grown backward into the posterior lobe; but these were entirely different in character from those composing the cellular mass, and serial sections showed no connection between them. In addition, a small chief cell adenoma, 3.5 mm. in diameter, was found in the anterior lobe.

Case 2. Colored male, aged 35 years. No reliable clinical data obtainable. Death was due to cerebral hemorrhage. The thymus was atrophied but small masses of thymic tissue were present in the fat behind the upper end of the sternum. Adrenals and thyroid apparently normal. The hypophysis was not enlarged. In the lateral and posterior part of the neurohypophysis was a mass, 0.25 mm. in diameter, composed of abnormal cells similar to those in the

other cases of this series. There was a slight backward growth of cells of the middle lobe into the neurohypophysis which had no connection with the mass of abnormal cells.

Case 3. Well nourished and well developed colored male, 32 years old. The chief symptoms complained of were shortness of breath on exertion and edema which became more and more marked until death. Necropsy revealed an insufficiency of the aortic valve due to malformation of one of its cusps, believed to have been congenital. Adrenals and thyroid normal in appearance. Approximately in the median plane of the posterior lobe of the hypophysis, was a mass of abnormal cells, the whole measuring 0.25 mm. in diameter. This mass differed from the others in this series only in that it was U-shaped and formed a sort of cap over a vascular loop made by an artery and vein.

Case 4. White female, aged 47 years. Markedly emaciated, and had the appearance of a woman 65 years old. She "drank a large amount of water," but no definite information was available as to the excretion of water and sodium chloride. She had syphilitic ulcers of the foot and tabes dorsalis. Wassermann test strongly positive. At necropsy all of the organs were more or less atrophied. In the posterior lobe of the hypophysis were three small groups of cells, somewhat smaller than those in the other cases here reported. These cells were embedded in a finely granular material which occupied the center of a small cavity; they had distinct outlines; their cytoplasm was clear and non-granular; their nuclei were large and stained rather palely. From the embedding material finely branching processes extended toward the walls of the cavities, which were lined with plump spindle-shaped cells. No blood vessels could be seen within the masses of cells.

Case 5. Well developed and well nourished colored man, 54 years of age. No note as to the quantity of urine passed. No edema. Death was due to lobar pneumonia complicated by a pneumococcic meningitis. The adrenals and thyroid showed no gross or microscopic pathologic changes. The testicles were atrophied and markedly pigmented. The hypophysis was not increased in size. In the postero-lateral quadrant of its posterior lobe was a millimeter-sized mass of cells with the morphology common to this group of cases. These cells were arranged in cords which ran more or less parallel

with each other and were separated by a fine supporting tissue. No vessels containing blood could be distinguished between the cords of cells.

Case 6. White male, aged 66 years. Clinical diagnosis, paralysis of the lower extremities due to pressure on the spinal cord by a tumor of the vertebrae. Well nourished but not obese. No edema. Quantity of urine ranged from 300 c.c. to 1125 c.c. per day; later developed incontinence and on some days as little as 60 c.c. of urine were collected. Nearly all of the specimens of urine contained a small amount of albumin and a few pus cells but no casts. Wassermann test negative. Necropsy showed a myeloma of the left 6th rib and of the 6th thoracic vertebra; hypostatic pneumonia; marked generalized arteriosclerosis; arteriosclerotic contracted kidneys. The adrenals showed no gross changes. The right lobe of the thyroid was enlarged and contained a mass which was diagnosed a tumor of the parathyroid. The testicles showed no gross abnormalities. The hypophysis was of normal size. In a postero-lateral quadrant of the neurohypophysis was a group of abnormal cells measuring 0.5 mm. in diameter. In addition this hypophysis showed a very marked fibrosis of the anterior lobe and a moderate backward growth of cells from the middle lobe into the neurohypophysis.

Case 7. White male, aged 52 years. Cause of death, influenza with bilateral bronchopneumonia. The hypophysis was of normal size. In a postero-lateral quadrant of its posterior lobe was a mass, 1 mm. in diameter, composed of large cells which tended to be arranged in long cords. A considerable amount of brownish pigment was present in this neurohypophysis, but none within the mass of cells.

Case 8. No clinical or other data available. At about the center of the posterior lobe of the hypophysis was a group of less than a dozen cells similar to those in the other cases of this series.

All of these patients showed in the posterior lobe of the hypophysis a fairly well circumscribed cellular mass, not encapsulated, and varying in size from less than 0.25 mm. to slightly more than 1 mm. in diameter. In all except one case this mass of cells was in one postero-lateral quadrant of the posterior lobe. They were not so near the median plane of the lobe as Priesel states was the case in his specimens.

These masses are composed of large, round, oval or polygonal cells which stain a bluish-purple and have abundant granular cytoplasm. The nuclei are round and usually eccentrically placed. In many cells they are deeply stained and pyknotic, or pale and apparently undergoing karyolysis. Some of the cells are entirely devoid of nuclei; others show shadowy nuclear remains. No nucleoli can be seen in any of the cells. Within the masses, the cells frequently occur in groups of 2 to 6 in direct contact with each other. The supporting tissue of these masses is scanty in amount and resembles that of the remainder of the posterior lobe, being characterized by an interlacing meshwork of very fine fibrils. In every case, except Case 4, many of the large cells give off from their peripheries, fine processes that take part in the formation of the intercellular fibrillar meshwork. Among these fibrils there are smaller cells with round and spindle-shaped nuclei. In a few places between the large cells of these masses, there are narrow spaces lined with long spindle cells with slender nuclei. Occasionally one or two red blood cells can be seen in these spaces. But the total quantity of blood in the vessels within these masses is very small. Pigment is absent from these masses of cells.

Two problems present themselves in connection with the lesion here described: (1) What effect, if any, has it on the function of the hypophysis; and (2) what is the nature of the mass and the origin of the cells that compose it?

1. Rasmussen [4] found that the posterior lobe of the hypophysis represents 18 per cent of the total organ. The average hypophysis weighs about 0.6 gram, and measures 13 × 10 × 6 mm. It is evident, therefore, that a mass of abnormal cells even so small as 0.25 mm. in diameter occupies a relatively considerable portion of the neurohypophysis. It would seem quite possible that such a mass, especially one of the larger ones, might produce some change in the function of the structure in which it occurs. The lesion may, therefore, have more than a mere theoretical interest.

Sternberg [2] believed that there was a definite causal relation between the lesion in the posterior lobe of the hypophysis and the marked retention of water and sodium chloride which was apparently the cause of the edema in his patient. Priesel [3] found no such association of retention and edema with the hypophyseal lesion in his cases. In our series, the only instance of edema was clearly

of cardiopathic origin. One patient (Case 6) did, apparently, eliminate relatively little water, but there was no edema, and no accurate information as to the intake of water. In none of our cases was there any record of excessive thirst and nothing resembling diabetes insipidus. While the clinical histories in some of our cases were unavoidably incomplete, none of the functional or bodily changes usually ascribed to lesions of the hypophysis was observed in any of our series. Nor was there encountered any structural or functional abnormality common to a sufficient number of these cases to warrant attributing it to the lesion here described. It is not impossible, however, that a more careful study of patients with this lesion in mind, may reveal some clinical manifestation ascribable to it.

2. Sternberg [2] and Priesel [3] believe that the masses of abnormal cells found in the posterior lobe of the hypophysis were definitely neoplastic in nature. The former classed the "growth" as a Choristoma; the latter as a "Progonoblastoma," a term used by Mathias [5] to designate a tumor which originates in "atavistic formations," "a developmental reversion in a phylogenetic zone of proliferation of an organ in some particular part of the body."

If this lesion is a true neoplasm, the origin of the cells which compose it is not easily determined. The neurohypophysis is derived from the central nervous system. It is composed, except for blood vessels and the scant accompanying connective tissue, chiefly if not wholly of glia cells. The presence of ganglion cells in this structure has been disputed. Benda [6] mentions cells which "resemble sympathetic ganglion cells." Neubert [7] described "pigmented, calcified and glycogen-containing ganglion cells" in the posterior lobe of the hypophysis. Kohn [8] has "never encountered ganglion cells in the hypophysis in man."

Sternberg [2] believes that the lesion is "a blastomatous growth of (ausgereifter) neurogenic elements," probably of glia cell origin. Cells somewhat similar to those described in these lesions have been observed in gliomas. They have been described as "giant glia cells" by Meyer,[9] "monster glia cells" by Storch,[10] and ganglion cells by Robertson.[11] Ribbert [12] has described a "spongioblastoma" of the brain in which there were large cells resembling in many respects those in the lesions here reported. (See Fig. 15, in Ribbert's article.) He believes that these "spongioblasts" are glia cells that have reverted to an earlier embryonic stage than is usually observed in

gliomas. Kohn [8] in Fig. 1 of Plate XV of his article pictures cells that are apparently identical in morphology with those in our cases, and classes them as "very large glia cells rich in protoplasm."

Priesel [3] believes that these "growths" originated from remnants of the cells lining the central tube of the infundibulum and were, therefore, of ependymal origin. His conclusion appears to have been based upon the fact that, in his cases, the masses of abnormal cells were in or near the sagittal plane of the neurohypophysis. In the majority of our cases the cell masses were situated in one of the posterolateral quadrants.

A further possible origin of these abnormal cells is from cells which are frequently found growing backward into the neurohypophysis from the middle lobe of the gland. This is unlikely (1) because the cells which compose these lesions are wholly unlike those which grow backward from the middle lobe; and (2) because serial sections fail to reveal any connection whatever between them.

Sternberg's patient was 65 years old. Of Priesel's cases, all but 2 were over 50 years old, and all but 5, over 60. The patients whose hypophyses we have examined in serial sections (over 200 in number) were of all ages, from the newly born to persons over 70 years old. Among these were the 8 cases here reported. Two of these patients were 32 and 35 years old respectively; one was 47, but looked to be 65; two were over 50, and two over 60 years old. It appears probable, therefore, that this lesion is more common in the later decades of life.

It is our opinion that the large cells in this lesion are changed glia cells, because many of them give off from their surfaces fine branching processes which take part in the formation of the intercellular fibrillar meshwork. Some of the cells in these lesions are undergoing degeneration or even necrosis, as is shown by the character of the cytoplasm and the nuclei described above. We cannot agree with Priesel that this lesion is a tumor originating in remains of the epithelium lining the central tube of the infundibulum, (1) because none of our cases showed columnar or cuboidal cells forming more or less well defined lumina, characteristic of ependymgliomas; and (2) because the cell masses in our cases were located well away from the median plane of the posterior lobe of the hypophysis.

Without being dogmatic, we are inclined to consider the lesion here described as a localized retrogressive change in the glia cells, the cause of which is not now apparent.

Summary

1. A peculiar lesion consisting of a group of large abnormal cells in the posterior lobe of the hypophysis is described.

2. This mass of cells, although actually small, is relatively large when compared with the size of the posterior lobe itself.

3. The nature of the lesion is not clear. It is probably not a neoplasm.

4. Nothing in the clinical histories or physical findings in any of our cases could be definitely associated etiologically with this lesion. Careful study of patients with this pathologic process in mind may, however, reveal some clinical manifestation ascribable to it.

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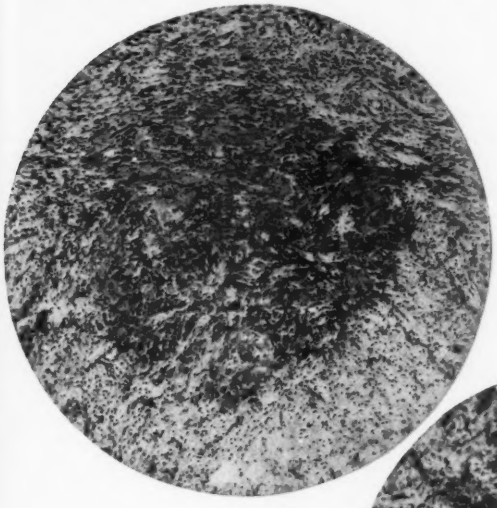
DESCRIPTION OF PLATE XLI

Fig. 1. From Case 7. One of the larger masses. x 45.
Fig. 2. From Case 1. x 82.
Fig. 3. From Case 3. Showing the relation of the mass of cells to blood vessels in this case. x 112.



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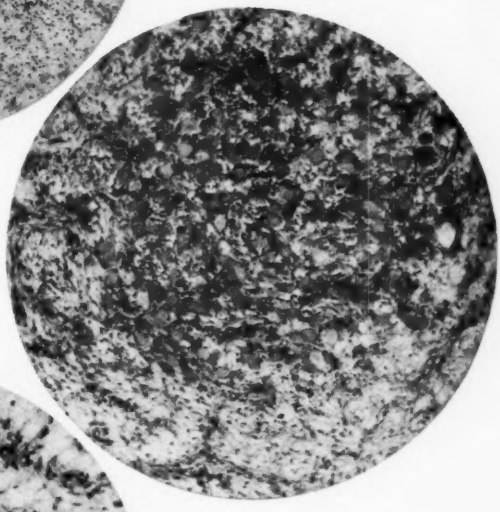

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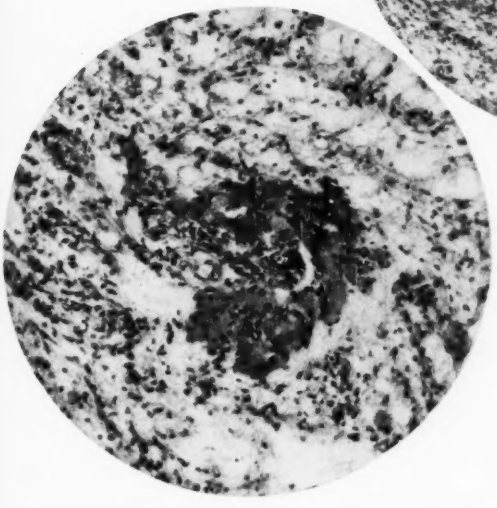

3

THE HYPERTROPHY OF THE SUBMAXILLARY GLAND IN THE ALBINO RAT, FOLLOWING PARATHYROIDECTOMY *

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The present report deals with the microscopic examination of the enlarged submaxillary glands of the parathyroidectomized albino rat. Hammett (1922) found that removal of the parathyroid glands from this animal resulted in a marked and valid increase in the size

TABLE 1

Nuclear counts in control (C) and parathyroidectomized (E) animals.

C	E	C	E	C	E	C	E	C	E	C	E	C	E
22	10	19	7	16	8	20	10	11	12	8	9	12	10
24	9	19	8	12	15	15	13	11	10	15	15	16	12
15	16	22	8	19	12	17	10	13	5	16	10	15	15
20	7	16	13	24	10	24	12	17	15	14	11	17	12
15	15	22	14	16	7	18	13	18	5	13	10	12	13
23	11	16	16	22	14	20	10	13	6	11	10	16	10
19	6	21	11	11	10	19	14	20	14	17	8	15	6
26	9	17	17	10	15	18	14	16	12	16	9	23	7
15	15	15	15	16	12	18	13	16	6	18	5	20	9
18	14	16	18	19	12	20	15	11	11	20	13	15	7
Average:													
19.7	11.2	18.3	12.7	16.5	11.5	18.9	12.4	14.6	9.6	14.8	10.0	16.1	10.1

Average for controls, per unit field 16.98
Average for parathyroidectomized, per unit field 11.07

of the submaxillary glands. This result did not follow thyroparathyroidectomy. As appears from Hammett's Table 1, the weight of the glands of the parathyroidectomized animal may exceed that of the litter control by as much as 100 per cent. An increase in weight is however not necessary. A decrease of as much as 21.2 per cent is recorded. In a later paper, Hammett (1923) presented

* Received for publication December 16, 1924.

evidence that the growth of the submaxillary glands of both sexes was increased over the normal by the lack of parathyroid secretion,

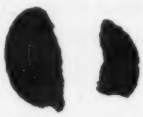

Fig. 1. Natural size. The larger submaxillary gland came from the parathyroidectomized animal: the smaller from the litter, sex and age control. The nuclear counts given in Table I were made from sections of these glands.

and noted that this effect was much more marked in the females. As an example may be cited a typical case where the glands came from two females of the same litter, killed at the same time. The larger gland weighed 0.7562 gr. and came from a parathyroidecto-

TABLE 2

Nuclear counts in control (C) and in thyro-parathyroidectomized (E) rats.

C	*C*	*C*	*E*	*E*	*E*
18	19	16	21	19	17
18	19	15	22	23	13
11	12	18	18	19	19
12	17	15	16	22	23
16	17	14	14	16	20
14	14	16	16	18	15
13	14	15	17	17	19
15	11	11	15	18	16
15	14	15	16	20	17
22	21	20	14	23	15
Average 15.4	15.8	15.5	16.9	19.5	17.4

Average for controls, per unit field 15.56
Average for thyro-parathyroidectomized, per unit field 17.93

mized animal weighing 167 grams. The smaller weighed 0.4240 gr. and came from the control weighing 217 grams. The larger gland weighed over 178 per cent of the smaller.

Material and methods. The submaxillary glands used in this study were very kindly furnished by Dr. Hammett. They were fixed in Bouin's or Zenker's fluid immediately after removal and

weighing. For each pair of glands from an experimental animal there was also taken for control a pair of glands from an animal of the same litter, sex and age. Glands from sixteen animals were prepared and studied: 6 parathyroidectomized, 3 thyro-parathyroidectomized, and 7 controls. In two instances the same control served for both types of experimental animals. The tissues were embedded in paraffin and cut serially at 5 μ. In some instances the plane of section was parallel to the cross-section of greatest area: and in other instances, parallel to the cross-section of least area. In mounting the sections, for economy, four or five contiguous sections were spread on the slide and then the next four or five discarded, and so on until all were spread. The "discarded" sections were usually employed for special stains for mucin and connective tissue.

In some instances sections from the gland of a parathyroidectomized animal and from that of the corresponding control, cut in similar planes, were mounted on the same slide. This of course ensured the same conditions of staining. The counts of nuclei recorded below were made from such slides.

The Submaxillary Gland of the Rat

Ranvier[13,14] and Loewenthal[8] have described the normal structure of this organ in the rat. At the cephalomesial aspect on the ventral surface a well-defined lobe is recognizable lying in a depression in the main mass of the gland. The relative size of the two lobes is very variable, not only in control animals of the same sex and litter but even in the right and left glands of the same individual. In making the nuclear counts, the smaller lobe or gland was ignored. Microscopically in the larger portion the lobules stand out more distinctly, presumably because they are separated from each other by broader trabeculae of connective tissue. The alveoli of the larger lobe are very small and belong to the so-called serous type. In the smaller lobe the alveoli are on the average larger and belong to the so-called mucous type.

The Submaxillary Gland in the Parathyroidectomized Rat

The description of Ranvier and of Loewenthal for the submaxillary of the normal rat is also valid for the glands of the parathyroidectomized and of the thyro-parathyroidectomized animals. No

qualitative differences could be noted in the histology of glands coming from controls and those from animals on which these operations had been performed. Sections cut in various planes and stained with Mallory's phosphotungstic acid hematoxylin, showed no recognizable differences in the quantity or distribution of white fibrous tissue. Likewise sections stained specifically for mucin (thionin, toluidin blue and muci-carmine) did nothing but confirm the observation of Ranvier that the larger lobe was devoid of mucous cells while the parenchyma of the smaller lobe was composed exclusively of the latter. No mitoses were seen. There was no lymphocytic infiltration or other sign of inflammation.

As just stated, simple inspection of stained sections made it seem unlikely that the enlarged size of the gland could be due to an increase in its connective tissue framework. This observation directed attention to the questions whether the parenchyma cells had increased in number or whether the individual cell had increased in size. The results reported below were obtained from glands (both experimental and control) which had so far as possible been subjected to the same histological technique. They were fixed in Zenker's fluid; an experimental and a control gland in the same bottle of the fixative and carried through all the subsequent steps together, cut the same thickness and mounted on the same slide. The animals were killed at the same time and after the same postprandial interval.

Attempts at measuring individual cells and of counting the number of cells in a unit area proved unsatisfactory. Resort was then had to counting the number of parenchyma nuclei in a unit area. The unit area was defined by a perforated piece of cardboard in the ocular. The areas were chosen quite haphazardly except that they were relatively avascular and free from larger strands or trabeculae of connective tissue. The same ocular, objective, and tube length were used throughout. In this way were counted the nuclei in ten fields at each of seven different levels (70 fields in all) of the submaxillary from the parathyroidectomized animal and in 70 comparable fields of the submaxillary from the corresponding control animal. The average number of nuclei per unit field for the parathyroidectomized rat was 11.07, while the average for the control was 16.98.

It is apparent that the individual cell of the submaxillary of the parathyroidectomized animal is considerably larger than that of the

control. Actually the ratio of their volumes is as 1.53 to 1 and the ratio of their linear dimensions would be as the cube root of these numbers or 1.15 + to 1. This means that if a particular dimension of the cell of the control animal measured 10μ, the similar dimension of the cell of the parathyroidectomized animal would measure a trifle over 11.5μ.

This increase in volume of the individual cell accounts at least in large part for the increased weight of the glands in the experimental animal. The weight of the gland from the control animal, from which these counts were made, was about 0.2120 gr. A gland, 1.53 larger, would weigh 0.3244 gr., while the actual weight of the gland of the parathyroidectomized animal weighed about 0.3781 gr.

The validity of the volume differences of the cells of the submaxillary gland of the parathyroidectomized animal and of its control, as specifically given above, is borne out by comparison with the nuclear counts obtained from glands of animals belonging to different series. The values obtained for the control and for the thyro-parathyroidectomized rats were not in all cases strictly comparable *inter se* because of technical differences, yet they indicate a cell appreciably smaller than the cell of the parathyroidectomized animal. The average nuclear count per unit field for 30 fields from the submaxillary of a thyro-parathyroidectomized animal was 17.93, while the corresponding count for the control was 15.56. These values are much nearer to that given above for the control (16.98) of the first series than are they to that for the parathyroidectomized of the first series (11.07). In other words, the parenchyma cell of the enlarged submaxillary of the parathyroidectomized is larger than those of the control or thyro-parathyroidectomized rats.

Summary and Conclusion

The enlargement of the submaxillary glands often observed in albino rats following parathyroidectomy (Hammett [3, 5]) is at least largely attributable to an increased volume of the individual parenchyma cell, i.e., a true hypertrophy. This was the only difference distinguishable between the submaxillaries of the parathyroidectomized animals and those of thyro-parathyroidectomized or of control animals.

Specifically, there was no histological evidence of any hyperemic,

fibrotic, inflammatory, proliferative, degenerative or infiltrative process.

A search of the literature has failed to reveal any other instance of submaxillary hypertrophy associated with a disturbance of parathyroid function.

Enlarged submaxillary (or other salivary) glands, described from the experimental or clinical point of view in connection with other endocrine disturbances (Horsley[6, 7]; Mikulicz [9]; Berthon [1]; Mohr [10]; Nagel [11]; Railliet [12]; Sprinzels [15]; Haemmerli [2]), apparently do not show the microscopic picture exhibited by the submaxillaries of parathyroidectomized rats.

Therefore, one is forced to the conclusion that the hypertrophy of the submaxillary glands which often follows parathyroidectomy in the albino rat is a condition *sui generis*. The data afforded by the present study seem to throw no light on the mechanism involved in this hypertrophy.

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HEMATOCELE OF THE WALL OF THE ATRIUM FOLLOWING CARDIAC PUNCTURE *

EDWIN W. SCHULTZ

(From the Department of Bacteriology and Experimental Pathology, Stanford University, California)

In another article [1] the writer has called attention to the ease and safety with which blood samples may be obtained from the dog by cardiac puncture. The chief source of danger is from puncturing the larger branches of the coronary vessels and thereby producing death from a hemopericardium. The animals usually die within a few minutes with the pericardial sac distended with blood. Death from this cause may be avoided by entering the thorax sufficiently low, so that by pushing the needle upward it will be sure to enter the apex of the heart. Here there are no large vessels. We have taken over 900 samples in this way with a mortality of less than one per cent on the basis of the number of samples taken. Several dogs were bled as high as forty-five times. We also have repeatedly bled the sheep by this method. For this purpose a 16 gauge, 3 inch, Luer needle is best. Etherization in an anesthetizing chamber also facilitates the procedure in the case of the sheep.

In addition to death from hemopericardium we have had one case of hemorrhage into the wall of the left atrium, which may be of sufficient interest to justify a brief report. Death followed a few minutes after the sample was obtained. On removing the heart it was found that a large rounded mass in the wall of the atrium had completely blocked the atrio-ventricular orifice. On section it was seen that a dissecting hemorrhage had split the wall into equal portions so that the hematoma which had formed was surrounded everywhere by a layer of tissue of about the same thickness. The mass was ellipsoidal in shape and measured 4 cm. in length and 2.4 cm. in thickness in the midportion. The hemorrhage had evidently taken place from a punctured vessel within the wall of the atrium.

* Received for publication October 21, 1924.

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DESCRIPTION OF PLATE XLII

Figs. 1 and 2 show appearance of hematocele before and after section.

Schultz Hematocele

THE HYDATID CYST *

THE MICRO-CHEMICAL REACTIONS OF THE HYDATID CYST WALL

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In a previous communication in 1923, one of us [1] dealt with a few of the staining reactions of hydatid cysts. Since then a more elaborate investigation into the micro-chemical reactions has been planned and the results are briefly presented here. The literature on this subject is extremely scanty, for apart from one or two short references, more or less indirect in their bearing, no work seems to have been recorded.

Echinococcus disease is fairly common in Australia, so much so that no differential diagnosis is considered to be complete unless due mention is made of it. The histological structure of the hydatid cyst appears, within certain limits, to be well known, and for many years the teaching of the Pathological Department of the University of Melbourne, under Professor Sir Harry Allen, has been very definite. The following description of the ordinary hydatid cyst, as found in man, may be taken as typical:

Two distinct portions are to be recognized:

1. The true cyst wall, consisting of: A. An outer layer or ectocyst, which is composed of many translucent, structureless laminae; B. An inner layer, the endocyst, or germinal layer, composed of a granular substance, presenting a cellular structure, but containing no nuclei. From this layer are derived the brood capsules, daughter and granddaughter cysts and scolices.

2. The adventitious capsule, which is simply the fibrous tissue reaction induced by the presence of the cyst, is composed of granulation tissue in all stages of development.

Daughter and granddaughter cysts arise by budding from the endocyst of the mother cyst, and have a laminated ectocyst and granular endocyst. Brood capsules have very delicate walls with no advanced specialization of layers, and may arise primarily from the mother cyst, or secondarily as buds within daughter or grand-

* Received for publication October 13, 1924.

daughter cysts. A scolex consists of an anterior rostellar portion with four suckers, and a double ring of hooklets, bounded behind by an hour-glass constriction, followed by a wide part which narrows rapidly into the stalk of attachment. The scolices are dotted with highly refracting particles of carbonate of lime, often double bordered or even laminated.[2] It has been the aim throughout this investigation to study the chief inorganic and organic constituents of the cyst wall by micro-chemical methods.

Methods used.

It is not proposed to describe in detail the various methods of staining. These have been dealt with, in part, elsewhere.[1] The following list presents briefly a summary of the methods employed:

Potassium	Macallum's technique.[3]
Sodium	Method of Rohdenburg and Krehbiel.[4]
Calcium	Macallum's technique.[3]
Iron:	
Inorganic iron	Prussian blue method.
Masked iron	Macallum's technique.[3]
Copper	Macallum's technique.[3]
Iodine	Justus's technique.[5]
Chlorides	Macallum's technique.[3]
Sulphates	Macallum's technique.[3]
Phosphates	Macallum's technique.[3]

Cholesterol. Fresh tissue, frozen and cut, is flooded with

formalin 30%	2 c.c.
sulphuric acid	6 c.c.

Cholesterol gives a brownish red color in 2 to 4 minutes.

Fats: Lipoids.

Sudan III. Ciaccio's method.[6]

Osmic acid (ordinary laboratory method).

Glycogen	Langhans's method.[7]
	Best's method.[8]

The hydatid tissue was obtained from the abattoirs and from the operating theatre, so that the chief varieties were studied. In all cases the tissue was at once frozen without any fixation and then stained. A section, stained with hematoxylin and eosin, was prepared from the same tissue and employed as a check. Reagents were prepared immediately before use.

Results.

1. *Potassium.* Section showed dense black staining of the whole of the hydatid wall, i.e., a strong reaction, both of the ectocyst and endocyst. Scolices were deeply stained also.
2. *Sodium.* Sections showed extensive patchy staining of the whole of hydatid wall. Scolices were deeply stained also.
3. *Calcium.* Marked diffuse reaction in all layers and in the scolices. Reaction varied with age of cyst, and extent of degeneration. In daughter and granddaughter cysts a slight reaction was obtained; in the mother cyst the reaction was vivid.
4. *Iron.*
   Inorganic iron. Pale green staining of ectocyst, i.e., a feeble reaction. Patchy blue staining of endocyst, deep blue staining of scolices, i.e., reaction more marked than that in ectocyst.
   Masked iron. Marked reaction throughout the whole of the cyst wall, as well as in the scolices. No special arrangement of the iron in the endocyst detected.
5. *Copper.* Faint reaction in the ectocyst, more marked in the endocyst. Distribution patchy.
6. *Iodine.* Faint reaction, more marked in endocyst and scolices.
7. *Chlorides.* Faint brown staining of whole of hydatid wall affecting the spaces between the laminae of the ectocyst and all structures of the endocyst. Scolices similarly affected.
8. *Sulphates.* No staining of ectocyst, deep black staining of endocyst involving all structures. Scolices deeply stained.
9. *Phosphates.* Very faint staining of both ectocyst and endocyst and scolices, irregularly distributed. Reaction rather more marked in endocyst than in ectocyst.
10. *Cholesterol.* Faint patchy reaction in endocyst and scolices.
11. *Fats: Lipoids.* All structures gave a reaction, including scolices. This varied considerably with the age of the tissue.
12. *Glycogen.* A positive reaction to both Langhans's and Best's tests was obtained.

A brief summary is included here of the reactions to certain of the special staining methods.

(*a*) HEMATOXYLIN AND EOSIN.

Ectocyst stains with eosin.

Endocyst stains with eosin, with patchy affinity for hematoxylin.

Scolices resemble the endocyst.

(*b*) VAN GIESON.

Fuchsinophil staining of ectocyst and endocyst although some staining with picric acid in patches was also present. Scolices stain with either or both stains.

(*c*) GIEMSA.

Light purple ectocyst. Irregular blue staining of endocyst and scolices.

(*d*) GRAM'S STAIN.

Eosinophil ectocyst.

Violet and pink areas in the endocyst.

Violet scolices.

(*e*) METHYL VIOLET.

Violet ectocyst.

Light violet endocyst.

Diffuse deep violet or blue scolices.

DISCUSSION

From a micro-chemical point of view, it seems that certain conclusions can be drawn as regards the constituents of the hydatid cyst wall.

In the first place, the ectocyst differs from the endocyst in its very slight reaction to the iron, phosphate, and chlorine tests, while there appears to be no reaction to the sulphate test.

In the second place, the ectocyst resembles the endocyst in its marked reactions to the potassium, calcium, and sodium tests, and in its feeble iodine test.

The endocyst is particularly rich in potassium, sodium, and calcium. It was noticed that in the more recent daughter and granddaughter cysts, the reactions to sodium and potassium were much more vivid than those in the case of the older mother cysts. On the other hand, the reaction to calcium became more evident with increase of age of the cyst. With degeneration, calcium salts practically replace the greater portion of the cyst wall, and may be de-

tected macroscopically as small or large earthy plaques. The ectocyst shows this infiltration at a slightly earlier period and to a greater extent than the endocyst.

In order to test this observation quantitatively, analyses of the sodium, potassium, and calcium content of the cyst wall have been made. Three types of cysts have been examined:

1. Daughter and granddaughter cysts.
2. Mature mother cysts.
3. Cysts which have just commenced to degenerate.

Number 1 represents the youngest variety of cyst, number 3 the oldest. The ordinary methods of analysis as described in Mellor's "Quantitative Analysis," were used. The results are as follows expressed in terms of percentage of undried weight:

	Calcium	Alkalies	Sodium	Potassium
1	0.465	0.678	0.607	0.071
2	0.959	0.455	0.389	0.066
3	1.968	0.252	0.152	0.100

From these figures it is plainly seen that the conclusion drawn from the micro-chemical tests is correct, with the exception that the potassium content of the degenerating cyst wall was somewhat higher than that in the young cyst. The hydatid cyst thus does not materially differ in this respect from certain other new growths, especially tumors, as shown by the work of Clowes and Frisbie [9] and of Beebe.[10] But there appears to be a considerable difference in the potassium content, which is so much less in the hydatid cyst wall. It will be seen that the marked potassium, sodium, and chlorine reactions go side by side with the high content of these substances in the hydatid fluid. It seems fairly certain that they are derived from the cyst wall, being secreted along with fluid into the cyst cavity.

A reaction for iron, both inorganic and organic, was well marked in the endocyst and scolices, less evident in the ectocyst, and this was associated with a faint, patchy reaction for copper. From its distribution, and from the much more vivid Prussian blue reaction, it seems likely that iron is present mainly in the form of inorganic compounds.

Cholesterol is often abundant in the fluid and a reaction to cholesterol in the cyst wall is given.

The glycogen reaction has been reinvestigated, using in addition Best's carmine method, and a confirmatory result obtained. It seems then, that glycogen is distributed throughout the whole of the cyst wall, in much the same way as fat.

Definite positive results were obtained, both with the Sudan III and the osmic acid methods, so that fats are present in the cyst wall and scolices. These reactions also become much more marked as the cyst grows older, and with degeneration there is extensive fatty change throughout the whole of the cyst wall, preceding the calcareous infiltration from the periphery of the ectocyst.

It has already been pointed out that an extensive fat reaction is sometimes found in the liver when a hydatid cyst is present.[1] This has been investigated in a number of cysts, and the conclusion reached that such a degeneration is by no means uncommon. The intensity of the fatty change depends to a large extent on the period over which the liver has been infected by the cyst. Apparently toxic substances are liberated during the growth of the cyst, giving rise to the leucocytic reaction as well as the degenerative changes, if continued over a long time.

With all methods of investigation, no indication of a nucleus could be obtained.

In regard to the special staining reactions, reference might be made to the affinity of the ectocyst for eosin. This is seen in the case of all composite staining methods in which eosin is made use of, e.g., Giemsa's method.

With all methods of staining, the scolices resemble closely the endocyst, a result which might be expected from the fact that the former are constantly developing from the latter.

The nature of the hydatid fluid will be the subject of a future paper.

Conclusions

1. The ectocyst of the hydatid cyst wall is (from micro-chemical observation) rich in potassium, sodium, and calcium, poor in iron, iodine, phosphates, and chlorides.

2. The endocyst shows well-marked reactions to tests for each of the above.

3. As the hydatid cyst grows older, the sodium and potassium

salts become less in quantity, while calcium salts, fats, neutral and unsaturated, and lipoids increase.

4. Iron is present mainly in the form of inorganic compounds.

5. Glycogen is distributed throughout the whole of the cyst wall, in much the same manner as fat.

6. Scolices closely resemble the endocyst in their micro-chemistry.

ACKNOWLEDGMENTS

We are indebted to Professor Sir Harry Allen and Acting-Professor F. L. Apperly for their interest and many suggestions.

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THE ORIGIN OF ADENOMATOUS GOITRE *

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There are two principal views concerning the origin of adenomatous goitre (struma nodosa). In the opinion of Beck,[1] Wölfler,[2] Ribbert,[3] etc., this condition arises from multiple embryonal cell nests scattered through the thyroid tissue, especially toward the periphery, which begin to enlarge from the time of puberty onward. According to the hypothesis of Virchow,[4] Hitzig,[5] Michaud,[6] etc., the development of the adenomatous nodules is from the adult thyroid tissue. From a microscopic study of over six hundred adenomatous goitres at Lakeside Hospital, the impression is gained that the latter view is correct.

Virchow in 1863 described the process as follows: "The cells of the acini increase by division in various portions of the acini. In this way solid plugs arise which push themselves outward, penetrate the soft interstitial tissue, again form new plugs and branch more and more. The interstitial tissue can be involved in the stimulation, proliferate and cut off portions of the plugs. . . . Later the plugs become hollowed out, fluid is secreted and they become active as acini. . . . If the development is uniform throughout the gland, the outer surface will appear comparatively smooth. . . . If it is unequal, a lobulated, nodular, knobby condition will result. The size of the nodules varies extraordinarily, according to the number of involved lobules, according to the degree of proliferation, and according to the secondary enlargement of the separate outgrowths and acini. Many nodules are barely pea-sized, others reach the size of a man's fist. If they lie at the periphery, they gradually push themselves away from the rest of the gland and at times come to lie quite independent of it. If they have a more central location, they squeeze the neighboring lobules together, compress them, so that these frequently form concentric zones about the nodules and finally lead to their atrophy. . . . In my opinion there are two en-

* Received for publication December 20, 1924.

tirely different orders of development: The capsular connective tissue formation arising from the old interstitial tissue and the landular hyperplasia arising from the old parenchyma."

According to Hitzig, "(1) The beginning of struma formation really depends upon proliferative processes in the normal colloid-containing and colloid-free glandular epithelium. (Virchow as against Wölfler): (2) The first definite appearances of struma nodosa are individual tubes of specifically differentiated epithelium which occupy the place of the normal tissue of the secondary lobules. (3) Furthermore, this tissue, more and more metaplastic, replaces the normal tissue of a secondary or even a primary lobule. (4) The lobules so altered, increase in volume, assume a spherical shape and displace the neighboring tissue. These masses constitute the smallest real struma nodules. (5) In the neighborhood, in a similar manner, altered lobules can form multilocular composite struma nodules by fusion (*a*) by the growth of the tubes through the septa, (*b*) by compression of lobules leaving the outer surface round, or finally (*c*) by growth and enlargement of compressed lobules surrounding central larger ones. (6) The metaplastic formation of the goitre tissue ends in the nodule when the boundaries of the primarily involved lobule are reached; then a growth of the nodule takes place from within itself with displacement of the surrounding tissue. (7) The diffuse goitre depends upon a similar proliferation in all lobules of the gland. (8) The nodular struma is brought about by a difference in growth energy of the different adjoining portions. (9) The greatest variety of transitions between the two kinds occurs."

In a microscopic study of over six hundred adenomatous goitres at Lakeside Hospital, adult thyroid tissue was observed apparently passing from its normal lobulation by gradual changes to circumscribed, encapsulated nodules histologically indistinguishable from adenomata.

In sections of thyroid glands showing varying degrees of hypertrophy and hyperplasia, more or less circumscribed areas are not infrequently found in which the hyperplasia is more marked than generally throughout the gland. These circumscribed areas may comprise one or more lobules or portions of lobules. In addition the repeated change in glands from a hyperplastic and hypertrophic condition to the colloid state apparently brings about in some an increase in stroma around and within lobules causing greater cir-

cumscription of lobules than normal, or division of lobules into smaller circumscribed units. It is possible that the changes just mentioned may underlie the initiation of adenomatous goitre, for, when hyperplasia occurs in these circumscribed areas (most marked usually at the periphery) they gradually increase in size and become spherical in form.

For a while the only encapsulation may be by the normal regional stroma. A definite capsule is gradually acquired by one of two processes apparently: (1) The compression of regional thyroid tissue with atrophy and replacement fibrosis thereof. (2) When the growing lobule returns to the colloid state, the older central acini usually take up much more colloid than the recently formed peripheral ones. The pressure exerted toward the periphery thereby, results in atrophy and replacement fibrosis of the outer acini with consequent increase in thickness of the perilobular stroma.

In adenomatous goitres there are many circumscribed, encapsulated nodules of this sort in which the relation of the acini to one another is similar to that in the normal thyroid gland. These nodules show the earliest transformation from normal thyroid tissue. It has not been possible, however, to decide whether a metaplasia occurs in the sense of Hitzig or whether this early change depends, as Virchow thought, merely upon the ordinary process of growth. In addition, in the ordinary adenomatous goitre, further stages of tumor formation are noted in which the central portions of the spherical lobules show congestion of the vessels, edema of the stroma, and separation of the acini from one another and from blood vessels. This congestion and edema of the lobule with disturbance in relationship of acini to one another and to blood vessels is a characteristic one and sharply marks off the adenomatous lobules from those of the normal thyroid gland. In addition to nodules of this type there are others varying in diameter up to 2 to 3 cm., showing gradations from these to tumors in which the congestion, edema, and separation of acini from one another and blood vessels are present from the center to the capsule, which at this stage is usually greatly thickened, sometimes to more than a millimeter. In this stage the histology of the nodules is indistinguishable from that of large single differentiated fetal adenomata. Furthermore, the same degenerative changes, such as central parenchymatous atrophy with great increase in edematous stroma with subsequent hyalinization

and even calcification of this tissue, are frequently present; hemorrhage and cyst formation also occur.

Conclusion

From a microscopic study of over six hundred adenomatous goitres, there is no evidence that this condition depends as contended by Beck, Wölfler, Ribbert, etc., upon a development after puberty of embryonic thyroid cell nests. On the other hand, the impression is gained from the study that adenomatous goitre arises, as suggested by Virchow, Hitzig, Michaud, etc., from adult thyroid tissue. Furthermore, there is evidence for their belief that it depends essentially upon an irregularity in the degree of proliferation of acini in different portions of the gland.

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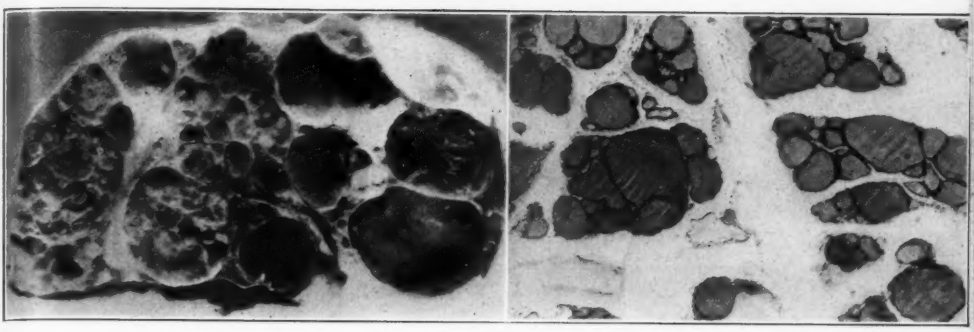
DESCRIPTION OF PLATES XLIII and XLIV

1. Gross photograph of adenomatous goitre.

   2–10. Photomicrographs illustrating possible origin of adenomatous goitre.
2. Lobules divided into smaller units, stroma increased, in return of thyroid from hyperplastic to colloid state.
3. Irregularity in degree of proliferation of acini in different portions of a lobule.
4. Early adenomatous transformation of lobule or portion of lobule (stage of hyperplasia). Lobule assuming spherical form. More marked hyperplasia at periphery. Normal relationship of acini to each other and to vessels.
5. Early adenomatous transformation of lobule or portion of lobule (colloid state). Older central acini contain more colloid than more recently formed peripheral ones. Normal relationship of acini to each other and to vessels.
6. Moderately advanced adenomatous transformation with separation of central acini from each other and from vessels.
7. Separation of acini from each other and from vessels due to congestion and edema.

8. Considerably advanced adenomatous transformation with separation of acini from each other and from vessels, reaching almost to capsule. Capsule well defined.
9. Advanced adenomatous transformation with separation of acini from each other and from vessels, reaching to capsule. Capsule thick.
10. (Formation of capsule.) Older central acini distended with colloid. Atrophy and replacement fibrosis of more recently formed peripheral acini.
11. Microphotograph of large single partially differentiated fetal adenoma (true tumor) with separation of acini from each other and from vessels due to congestion and edema.
12. Fetal adenoma (same as 11) with separation of acini from each other and from vessels, reaching to capsule. Capsule thick.



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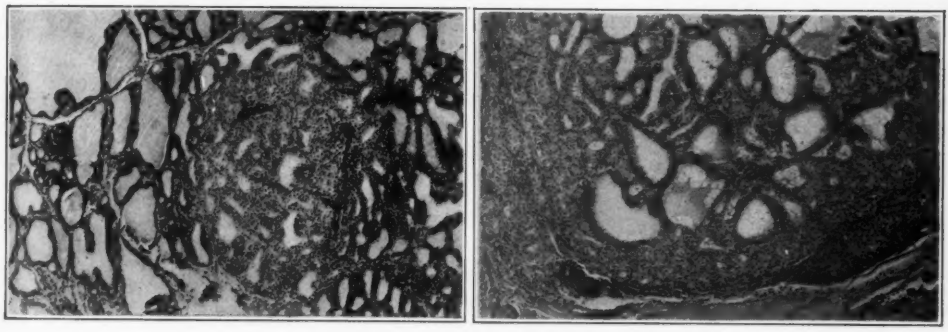

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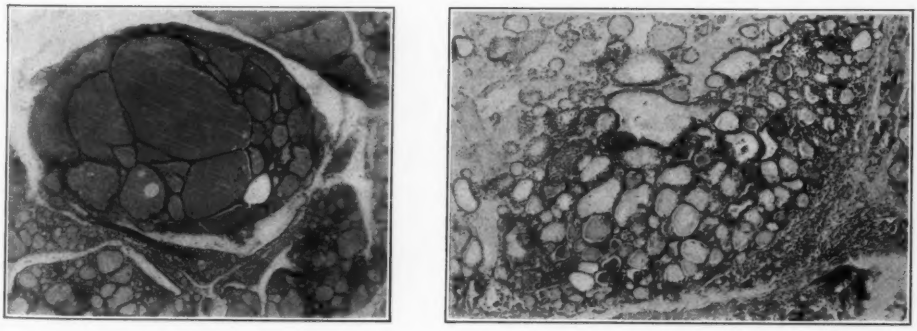

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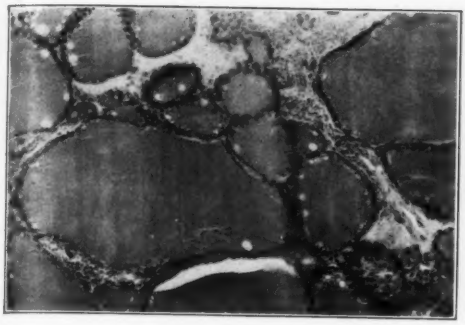
Kline Adenomatous goitre



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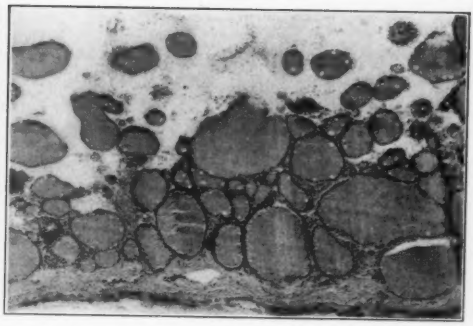

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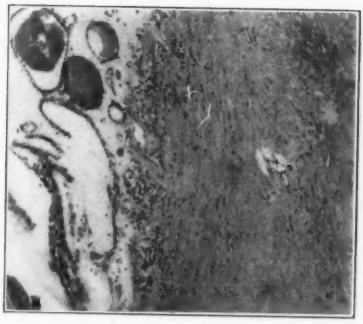

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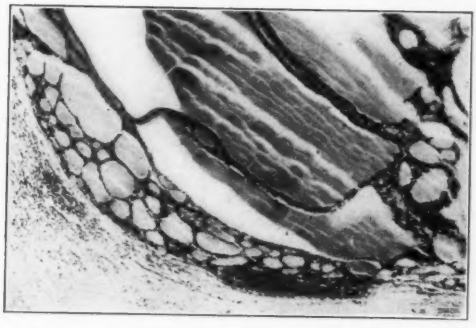

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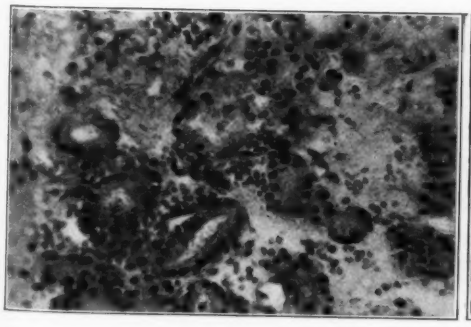

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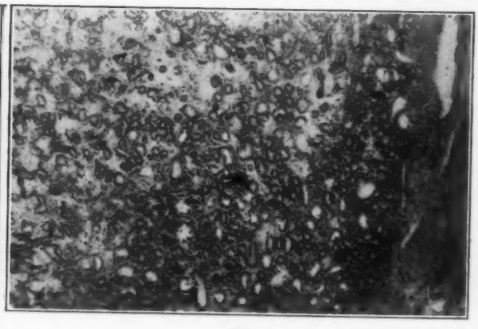

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Kline

Adenomatous goitre

THE ETIOLOGY OF ACUTE APPENDICITIS *

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In spite of the frequent occurrence of appendicitis and the thorough clinical and pathological understanding of the disease, there is still considerable discussion as to its etiology. Aschoff [1] believes the streptococcus, either alone or associated with a slender, curved Gram positive bacillus, to be the causal agent. McWilliams [2] took swabs from the appendicial serosa at the time of operation and cultured the swabs. Of 288, 57 per cent yielded B. coli, 19 per cent B. coli and Streptococcus, and 8.3 per cent a streptococcus alone. Kelley [3] found B. coli in the majority of pathological appendices he cultured.

Most of the work done on this subject has been purely bacteriological or purely histological. The advantage of combining these two methods of study is obvious, and the procedures described below were adopted as best suited to demonstrate the presence of organisms and to provide an accurate check against one another.

Methods. The excised appendix was dropped by the surgeon into a sterile Petri dish and then carried to the laboratory, care being taken to avoid contamination with material from the lumen. Occasionally when appendices were removed at night a few hours elapsed before they were examined. However, these were kept cold, and controls showed very little change to occur.

The serosa, and exudate covering it, if any, was dissected off with sterile precautions, over an area about 1 cm. square. That portion of muscularis thus exposed was removed, taking so far as possible the entire thickness of the muscle without breaking through into the mucosa. Finally a bit of mucosa together with some of the contents of the lumen were removed.

Each of these pieces of tissue was smeared over the surface of a blood agar plate, and then divided, one portion being dropped into a tube of peptone broth and the other into a tube of previously heated and cooled peptone broth which was overlaid with sterile

* Received for publication January 5, 1925.

petrolatum after inoculation. The organisms which developed under these various conditions were studied and classified.

The appendix was fixed *in toto* in Zenker's fluid and paraffin sections made from various portions, always including the immediate vicinity of the tissue taken for culture. Duplicate sets of sections were stained with eosin-methylene blue and by the Gram-Weigert method.

The cultural findings were then checked against the histological. No obvious discrepancies were encountered, though naturally organisms were sometimes not found in the sections when cultures showed them to be present.

Material. A large number of appendices removed by members of the Surgical Staff of the Boston City Hospital from cases diagnosed as acute appendicitis were examined and one hundred selected. Symptoms had existed in these cases from a few hours to a few days, from 12 to 48 hours in the majority. Most appendices showing little or no evidence of inflammation were discarded, though a few were included in the series for control purposes.

Of the 100 appendices, 66 were diagnosed histologically as acute appendicitis, 4 as acute periappendicitis, 15 as healing appendicitis, 1 as healed appendicitis with obliteration of the lumen with recurrent healing appendicitis, 1 as chronic periappendicitis (tuberculous), 10 as healed appendicitis, of which one showed healed fibrous tubercles, 2 as healed appendicitis with obliteration of the lumen, and 1 as healed appendicitis with acute inflammation of a nearby mesenteric lymph node.

None showed evidence of parasitic worms in the lumen. From casual observations in this laboratory, we have the impression that parasitic worms very rarely are found in acute appendicitis, though present in about 2 per cent of negative or healed appendices.

We regard the following as the minimum requirements for a diagnosis of the various conditions:

for acute appendicitis, pus in the lumen and some evidence of inflammation in the wall;

for healing appendicitis, fairly numerous eosinophiles in the muscularis, also foci of lymphocytes;

for healed appendicitis, focal collections of lymphocytes in the muscularis or serosa, either inside or outside lymphatics.

The diagnosis chronic appendicitis is not made. The dividing line between acute and healing appendicitis is not very clear-cut, but the lesion is called healing when there is a predominance of eosinophiles over polymorphonuclear leucocytes in the exudate and evidence of proliferation of fibroblasts. The presence of eosinophiles in the mucosa is considered normal.

Observations. The bacteriological findings in the 66 acutely inflamed appendices show B. coli and Streptococcus hemolyticus, with or without B. coli, to be most frequently encountered. In Table 1 are presented the bacteriological results. In two instances organisms

TABLE 1

Organisms present in the wall in acute appendicitis. 66 cases

	Serosa	Muscularis	Mucosa	Total
B. coli communis	14	17	18	18
B. coli communior	7	7	7	7
Streptococcus hemolyticus	5	5	5	6
Streptococcus hemolyticus + B. coli	9	9	9	9
Proteus vulgaris	4	4	5	5
B. pyocyaneus	4	4	4	4
B. pyocyaneus + B. coli	1	1	1	1
Streptococcus viridans	3	2	3	3
Streptococcus viridans + B. coli	1	1	1	1
Staphylococcus albus + B. coli	1	1	1	1
Pneumococcus type I	1	1	1	1
Streptococcus (non-hemolytic)	0	0	0	1
No growth	..	..	..	9

were found in the lumen only — Streptococcus hemolyticus in one case and a non-hemolytic streptococcus in the other. No typhoid appendices were encountered, in spite of fairly numerous cases of typhoid fever admitted to the hospital.

The four cases of acute periappendicitis all occurred in females. From the serosa of two of the appendices Pneumococcus type I was recovered. One of these two cases, a girl of five years old, developed a lobar pneumonia four days after operation and Pneumococcus type I was recovered from the sputum. Another of the cases of acute periappendicitis yielded Streptococcus viridans, and in the last (who gave a history of pelvic inflammation) the gonococcus was found to be the causal agent.

Of the sixteen cases of healing appendicitis, one showed B. coli and one Streptococcus viridans, both present in the serosa and not in

the muscularis. These two appendices were not far removed from the acute stage of the process. The remainder showed no growth. Organisms were found in only one of the appendices in the healed stage occurring throughout the wall, and that one was left twelve hours at room temperature to determine how far organisms in the lumen would grow out into the wall.

A lymph node accompanying one appendix, which was diagnosed as healed, showed evidence of acute inflammation, and B. coli was recovered from the node.

Discussion. The earliest stage of the lesion found in these acutely inflamed appendices is necrosis and slight ulceration of the mucosa, not in those portions nearest the center of the lumen, as would be expected if a bacterial poison present in the lumen were causing the change, but down in the folds, somewhat suggesting the early inflammatory changes in the crypts of the tonsil. All of the organisms found in the lesions, with the exception of a pneumococcus, are fairly common in the intestinal flora. There is little need, therefore, to hypothecate a hematogenous origin of the infection. The character and location of the early lesions are decidedly in favor of an invasion from the lumen.

The peristaltic action of the muscularis of the appendix is probably of great importance in keeping the contents of the lumen in motion, and any interference with this action, whether functional or structural, due perhaps to scarring from previous attacks, will favor the accumulation of bacteria in large numbers and ultimately permit sufficient concentration of bacterial products to cause necrosis of adjacent epithelium. Obstruction of the lumen, whether by kinking, partial obliteration by scarring, or the presence of fecoliths, would have the same effect.

The occasional appendices which showed no organisms either on culture or histologic examination and yet were acutely inflamed can probably be explained by the destruction of the invading organisms by the defense mechanism of the body, but only after they had done serious damage.

One striking feature is the similarity of the exudate in all acutely inflamed appendices, in spite of the various organisms calling it out. In this small series, at least, it is impossible to pick out any one organism as tending to produce a given type of appendicitis. The

factors giving rise to a phlegmonous inflammation as against an ulcerative, for example, are not etiological but must be sought for in anatomical peculiarities or in the locations of the invading organisms. The mucosa is apparently much more resistant than the muscularis or serosa, and the infection often spreads in these layers outside the mucosa, so it is not unusual to find a lesion that appears phlegmonous in one section to be ulcerative in another. I have the impression, however, that the exudate is decidedly more hemorrhagic and necrosis more widespread in those appendices in which Streptococcus hemolyticus was found, but no definite line can be drawn. Such findings are, however, entirely in keeping with streptococcus lesions elsewhere.

The same similarity holds for the healing process. The predominance of eosinophiles in the exudate, once repair begins, appears to be a peculiarity of the appendix, as they are rarely encountered in any numbers in other portions of the intestinal tract. What the reason for their constant occurrence in healing appendicitis may be I have been unable to determine.

As mentioned above, the time between onset of symptoms and removal of the appendix varied considerably in these cases. As a rule there was a fairly close correspondence between the duration of symptoms and the pathological findings. The cases operated on within a few hours of the onset usually showed only a small area of necrosis and ulceration with leucocytic infiltration. From then up to forty-eight or seventy-two hours there is no evidence of repair other than slight proliferation of fibroblasts, which is rather a response to injury than a definite attempt at repair. Those appendices removed four to eight days after onset of symptoms generally showed active fibroblastic proliferation, a predominance of eosinophiles in the exudate, and mitoses in the glands, if any epithelium were left. A few showed no evidence of healing up to the sixth day, and one was completely gangrenous.

Those cases diagnosed pathologically as healed appendicitis usually gave a history of one or more previous attacks dating back weeks to months, and sudden pain of a few hours duration immediately preceding operation.

Though the cases are not included in this series, we have recently had evidence that acute appendicitis can exist without noteworthy symptoms. One appendix removed routinely with chocolate cysts

of the ovary and vaginal wall was found to have pus in the lumen with necrosis, ulceration and leucocytic infiltration of the mucosa at several points and slight infiltration of the muscularis. Another removed with a fibroid uterus was even more inflamed. Neither of these cases had given the surgeon reason to suspect any acute abdominal condition.

Summary and Conclusions

1. The bacteriological and histological results from a study of 66 cases of acute appendicitis show B. coli to be the organism most commonly encountered, being found alone in 25 appendices and combined with other organisms in 12. Streptococcus hemolyticus occurred alone in 6 and combined with colon in 9. Proteus vulgaris and B. pyocyaneus were each found five times. There was a scattering of other organisms.

2. All early lesions found were at the margin of the lumen.

3. The inflammatory reaction does not vary with the organism found in the lesion, although it is perhaps more hemorrhagic in those cases where Streptococcus hemolyticus was recovered.

4. In this series of cases the delicate Gram-positive bacillus described by Aschoff was not found in the lesion and only rarely in the contents of the lumen.

5. One case of Pneumococcus type I periappendicitis in a five-year-old girl was followed in four days by lobar pneumonia, also due to Pneumococcus type I.

6. The evidence is against a hematogenous origin of acute appendicitis.

7. Acute appendicitis is not a specific disease due to one type of organism, a streptococcus as sometimes maintained. Like acute tonsillitis, acute endocarditis and similar infectious lesions, it may be caused by a variety of organisms.

8. Acute appendicitis may occur without symptoms.

I am indebted to Dr. R. C. McLeod of this laboratory for help in preparing and examining some of the specimens, and to the Surgical Services of the Boston City Hospital for the material used.

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